AR201-14072

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

November 14, 2002

Via US Mail and E-mail

Christine Todd Whitman
1101A
USEPA Headquarters
Ariel Rios Building, Room #3000
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Re: Hydroquinone Precursors and Derivatives Panel
Consortium No.
HPV Chemical Challenge Program Submission
Diisopropylbenzene Category Justification and Testing Rationale

Dear Governor Whitman:

The Hydroquinone Precursors and Derivatives Panel (HQPD) DIPB Task Force of the American Chemistry Council is pleased to submit the subject documents to EPA's HPV Chemical Challenge Program (Program) as our test plan for a category covering three chemicals (CAS Nos. 99-62-7, 100-18-5, and 25321-09-9). The DIPB Task Force includes the following member companies that are sponsoring these chemicals under the Voluntary HPV Chemical Challenge Program: Eastman Chemical Company, Georgia Gulf Corporation, Goodyear Rubber and Tire Company, and Koch Specialty Chemical Company.

This submission includes the following documents:

- The Test Plan for Diisopropylbenzene Category
- Appendix I

This submission is also being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtk@epa.gov

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

If you require additional information, please contact F.J. "Sonny" Maher, HQPD Panel Manager at (703) 741-5605 or sonny_maher@americanchemistry.com.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Attachments

cc: DIBP Task Force
Steven Russell, ACC
Jim Keith, ACC

AR201-14072A

HIGH PRODUCTION VOLUME (HPV) CHALLENGE PROGRAM

DIISOPROPYLBENZENE CATEGORY

TEST PLAN

m-DIISOPROPYLBENZENE [CAS Registry No. 99-62-7]
p-DIISOPROPYLBENZENE [CAS Registry No. 100-18-5]
DIISOPROPYLBENZENE [CAS Registry No. 25321-09-9]

PREPARED BY:

MEMBER COMPANIES OF THE AMERICAN CHEMISTRY COUNCIL'S
HYDROQUINONE PRECURSORS AND DERIVATIVES PANEL
DIISOPROPYLBENZENE TASK FORCE

EASTMAN CHEMICAL COMPANY
GEORGIA GULF CORPORATION
GOODYEAR RUBBER AND TIRE COMPANY
KOCH SPECIALTY CHEMICAL COMPANY; a division of KOCH PETROLEUM GROUP, L.P.

October 3, 2002

TABLE OF CONTENTS

OVERVIEW

The diisopropylbenzene (DIPB) category consists of a group of three chemicals consisting of CAS Registry Numbers 99-62-7, 100-18-5, and 25321-09-9. Two of the three members, meta-DIPB and para-DIPB, are pure isomers while the third member is a Class II chemical consisting of a mixture of all three ortho-, meta-, and para-DIPB isomers (xDIPB). In preparing this test plan, the Hydroquinone Precursors and Derivatives Panel has given careful consideration to the principles contained in the letter the EPA sent to all HPV Challenge Program participants on October 14, 1999. As directed by EPA in that letter, the Panel has sought to maximize the use of existing data for scientifically appropriate related chemicals and structure-activity-relationships. Additionally, and also as directed in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than use a rote checklist approach. It is the intent of the Panel to fulfill all the Screening Information Data Set (SIDS) endpoints of the HPV program through the use of data that are already in existence. For the DIPB category, this data set consist of results from studies conducted specifically on either one of the pure meta- and or para-isomers, or with results from studies conducted on xDIPB (the mixture of all three isomers). In addition, some endpoints have been completed through the utilization of data from studies conducted on structurally similar compounds and from modeling programs accepted by the EPA. The Panel believes these data are adequate to satisfy the requirements of the HPV program without need for the conduct of any new or additional tests.

SUMMARY OF TEST PLAN AND DATA

The diisopropylbenzene (DIPB) category consists of a group of three chemicals consisting of CAS Registry Numbers 99-62-7, 100-18-5, and 25321-09-9. Two of the three members, meta-DIPB and para-DIPB, are pure isomers while the third member is a Class II chemical consisting of a mixture of all three ortho-, meta-, and para-DIPB isomers (xDIPB). At this time the sole commercial use for the individual pure DIPB isomers are as industrial intermediates in the synthesis of other chemicals. Similarly, commercial applications for xDIPB are primarily as a raw material for chemical manufacture; however, it is also used as a component in an industrial cleaning formulation. Therefore, no isomer of DIPB is known to be distributed in commerce for any non-industrial uses or applications in consumer products. Purposeful production of DIPB occurs through the alkylation of benzene with propylene in the presence of a catalyst, followed by distillation to meet purity specifications. Some mixed DIPB is formed as a
by-product in the manufacture of cumene (mono-isopropylbenzene) where part of the cumene is further alkylated with available propylene to form xDIPB.

In general, the individual meta- and para-isomers are quite pure when sold (mDIPB purity is >95% and pDIPB is >99%), with the primary contaminants consisting of various other DIPB isomers. xDIPB may contain small amounts of cumene and other aromatic hydrocarbon impurities. They are all manufactured and transported in closed systems and have a very limited number of customers who also handle them in closed systems. Occupational exposure to DIPBs is minimized by the manner in which they are manufactured and through good industrial hygiene practices. Routine exposure to the general population is not anticipated. Significant environmental exposures from their manufacture and use are unlikely except under conditions of a spill incident.

The three DIPB CAS numbers that constitute the DIPB category the Panel is submitting are obviously very similar from a structural standpoint as they are all isomers of the same compound and possess nearly identical physical-chemical properties. In addition, all available hazard data indicate these substances induce a similar toxicological profile following either acute or repeated exposures, with the liver and kidney being the primary target organs. Accordingly, the Panel believes that data generated on any one of the individual isomers as well as data from studies conducted on the mixture itself (xDIPB) can be used interchangeably in the evaluation of their environmental fate, ecotoxicity, and mammalian toxicity potentials (See Table 1).

In addition to the interchangeable use of data from the various DIPB compounds to substitute for each other, there was a need for the utilization of data from various other short chain mono- and di-alkylated benzene compounds. Specifically, these other surrogates consisted of either: isopropylbenzene, ethylbenzene, and various diethylbenzene isomers (ortho-, meta-, and para-). These other alkylbenzene compounds were used to assess hydrolytic degradation potential, ability to impact algae growth, and in the determination of the potential for DIPB to induce reproductive and/or developmental toxicity. The Panel believes the use of these compounds as surrogates is valid based on their structural, physical-chemical, and metabolic similarities to DIPB. DIPB and the aforementioned surrogates are predominantly metabolized via oxidation reactions on the alkyl side chain followed by conjugation reactions. In addition, these compounds share with the various DIPBs a similar acute toxicity potential and target organ specificity (liver and kidney) following repeated exposure (See Table 1).

Data assessing the various physical-chemical properties (melting point, boiling point, vapor pressure, partition coefficient, and water solubility) for the different DIPB isomers were obtained from either reputable textbooks, actual study data, or from computer estimation modeling programs accepted by EPA and found in EPIWIN (Version 1.2, Syracuse Research Corporation, Syracuse, NY). These data indicate that the DIPBs are liquids at room temperature with a low potential to volatilize. They are essentially insoluble in water but highly soluble in organic solvents. The quality of the available information meets the requirements of the various endpoints to preclude the need for any additional physical and chemical properties testing.

Data from studies conducted on the various DIPBs, structurally similar compounds, or estimation modeling programs accepted by EPA were available, and of sufficient quality to complete the assessment of all the environmental fate endpoints (photodegradation, biodegradation, stability in water, and fugacity). Overall, due to its low volatility, fugacity estimations predict that DIPB will distribute primarily to soil and water. Available data indicate DIPB is not readily degraded or even soluble in these two media. Although its release into the environment would primarily occur through fugitive emissions and evaporative mechanisms, atmospheric hydroxyl radicals are

predicted to readily break down the molecule. In addition, data from a study assessing its volatility from water demonstrated that there is a 100% loss from an aqueous saturated solution after 96 hours (Unpublished 1986 Kodak report).

The toxic potential of DIPB to fish and aquatic invertebrates were determined through studies using both mDIPB and pDIPB, and its potential to affect algae growth was evaluated through the use of modeling. Modeling results were then compared to actual studies conducted on two structurally similar surrogate compounds (isopropylbenzene and 1,4-diethylbenzene). In total, these data demonstrate DIPBs are not toxic to these particular organisms at concentrations that are either at, or near, their saturation point in water. Coupled with the extremely low water solubility of DIPBs, the potential for exposure of these substances to aqueous organisms is also very unlikely due to its primary use as an industrial intermediate.

The potential to induce toxicity in mammalian species following acute oral exposures is very low and, as previously noted, the potential for human exposure is believed to be quite limited. The results of studies conducted on both isomers and the mixture indicate these materials are only slightly toxic with LD50 values ranging from >3200 mg/kg to >5000 mg/kg. Data were available on all three CAS numbers evaluating their effects following repeated oral exposures with exposure durations ranging from 12 to 28 days. Results of these studies demonstrated that the pure isomers and the mixture induce effects in the stomach (nonspecific irritation), liver (weight increase in absence of any changes in morphological appearance) and kidney (hyaline droplet accumulation). These changes were most prominent at the highest dose levels. Such effects in the liver are often considered as an adaptive response by the animals to the high dose levels of chemical they are receiving. This effect reversed itself following a 14-day recovery period. The changes noted in the kidney were specific to males and are interpreted to be due to accumulation of alpha-2u-globulin protein. Accumulation of this protein in the kidney and its pathological consequences are unique to the rat species and are not believed to be of concern for humans who lack this protein. Evidence of a localized gastric irritation was also noted in some studies. This effect is believed to be due to the manner in which the animals received the test material (i.e., as a single large oral bolus), resulting in a small surface area of tissue exposed to a high concentration of test material. Several mono- and di-alkylbenzene compounds were utilized as structural surrogates to assess the potential of DIPB to induce developmental and reproductive toxicity. The Panel utilized a well-recognized reproductive toxicology expert to assess the validity of this approach. It was the opinion of this expert that "additional studies on analogs or indeed, the diisopropylbenzene isomers themselves, would only serve the limited objective of confirming the absence of hazard to reproductive and developmental toxicity at reasonable oral or inhalational exposures." Results from several different studies conducted on DIPB as a mixed isomer indicate these compounds do not induce genotoxicity.

In conclusion, the Panel believes that it has completed adequate assessment and summarization of all the Screening Information Data Set (SIDS) endpoints to satisfy the requirements of the HPV program without need for the conduct of any new or additional tests. This data set consists of results from studies conducted specifically on either the pure meta- and/or para-DIPB isomers themselves, or with results from studies conducted with the mixed isomers. Where appropriate, some endpoints have been fulfilled through the utilization of data from studies conducted on structurally similar compounds and from modeling programs accepted by the EPA. The summarized data indicate that these chemicals, as used in commerce, constitute a low risk to both workers and the general population.

TEST PLAN FOR DIISOPROPYLBENZENES

I. Category Justification and Use of Surrogate Data

As a means to reduce the number of tests that may be conducted, the EPA allows for the use of categories to group together chemicals that are structurally similar to characterize specific SIDS endpoints (USEPA 1999a). Obviously, the chemicals that comprise the three CAS numbers that form our category are structurally similar as they are all isomers of DIPB. As seen in Table 1 below, all three CAS numbers have very similar physical-chemical properties, and induce a similar toxicological profile following either acute or repeated exposure, with the liver and kidney being the major target organs. Accordingly, the Panel believes that data from an individual pure isomer or data from studies conducted on the entire mixture of all isomers (xDIPB) may be used interchangeably to complete the hazard assessment for any specific endpoint.

In addition to the interchangeable use of data from different DIPB isomers to complete some endpoints, there is also a need for the use of surrogate data from various other short chain mono- and di-alkylated benzene compounds to assess the potential for DIPB to induce reproductive and developmental toxicity. Specifically, the compounds isopropylbenzene (cumene), ethylbenzene, o-, m-, and p-diethylbenzene are believed to meet the criteria needed to allow for their use as surrogates in assessing reproductive and developmental toxicity. As is readily seen below in Table 1, these compounds are all very similar in structure, physical-chemical properties, acute toxicity potential, as well as target organ specificity following repeated exposures.

Results of metabolism studies conducted on various alkylated benzene compounds indicate that these types of compounds undergo similar routes of metabolic reactions. These reactions are characterized by phase I biotransformations on the alkyl side-chain to form alcohols and/or carboxylic acids. These metabolites are eventually eliminated in the urine following phase II transformations as conjugates of glucuronic acid or glycine (Williams, 1959, Bakke and Scheline, 1970). With ethylbenzene, the principal metabolic pathway in rats is believed to be the same as in humans (Climie *et al*, 1983), and its metabolites in animals has been shown to be similar without regard to route of exposure (Climie *et al*, 1983). Similarly with cumene, very similar rates of metabolism of the chemical and routes of elimination were observed for oral and inhalation exposures in animals (Bushy Run Research Centre, 1989c). Unfortunately, at this time metabolic data specifically on DIPBs are not available. While it is possible that hydroxylation reactions on the aromatic ring may take place to form phenols, there is no evidence reported that these types of compounds would undergo complete dealkylation reactions in order to form benzene. Thus, overall, the question of toxicity induced by the metabolic hydroxylation to phenols is mitigated owing to the small quantities of metabolites involved and partly to their subsequent rather quick conversion to glucuronides and etheral sulfates (Bakke and Scheline, 1970).

The Panel sought an independent review by Mr. James Schardein, an independent consultant formerly employed by WIL Research Laboratories, Inc., and expert in reproductive toxicology, to determine the appropriateness of data from surrogate chemicals to complete the reproductive and developmental toxicity endpoints. Mr. Schardein concluded that the approach the Panel took in regard to utilizing surrogates for these specific endpoints was appropriate and that the data from the surrogates was of sufficient quality to fulfill the required endpoints. The following are excerpts from Mr. Schardein's review (Attachment I).

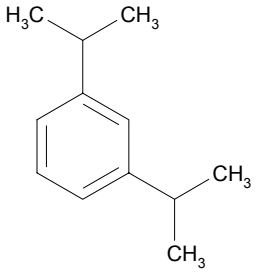
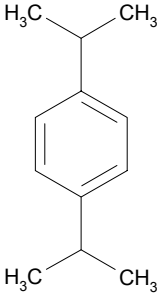
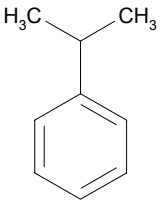
"I consider the chemicals selected to serve as surrogates to be a valid approach in fulfilling the reproductive/developmental endpoint evaluation for the diisopropylbenzenes, since acceptable data exists on these chemicals (see following)."

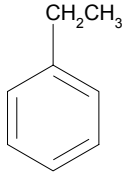
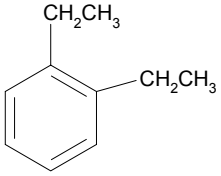
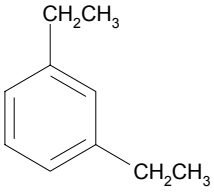
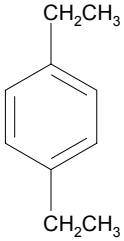
"The existent *developmental toxicity* studies in one (oral route) and three (inhalation route) species with the alkylbenzene analogs demonstrate quite convincingly the potential for developmental toxicity in laboratory species. The SIDS requirement is, in fact, for one species testing. In my judgment, no further developmental toxicity studies on the candidate diisopropylbenzene chemicals are needed, as the data on the surrogates suffices. The present data available for interpretation are fully adequate; no data gaps are evident, and additional studies would add little to the database already gleaned from the completed studies with respect to effects on development, by either route of exposure, oral or inhalation. The more critically conducted and robust studies evaluated (Bushy Run Research Centre, 1989; Saillenfait et al, 1999) on the 1,2-DEB and cumene analogs indicate embryotoxicity at maternally toxic levels, but no teratogenicity."

"The results with the *reproductive toxicity* studies conducted on the diisopropylbenzene analogs are less perfect. In fact, only the data from the study conducted on 1,4-DEB is suitable for adequate characterization of the *conventional* reproductive toxicity assessment of diisopropylbenzene analogs. The remaining two studies, conducted on cumene and ethylbenzene, were not conceived with the objective of fully characterizing their reproductive toxicity potential. However, it cannot be stressed too emphatically, that the studies on the latter two analogs provide much valuable information on the reproductive process in other ways. In both the cumene 90-day inhalation toxicity study in rats and in the ethylbenzene 28-day inhalation toxicity study in three species (see Table 4), alternative study designs that have been considered in the past as acceptable in the SIDS testing scheme, are more than adequate, since there was assessment of the reproductive organs (without mating trial). No toxicity was reported in either study with respect to histopathology of the testes, testicular weight, or the process of spermatogenesis (as evidenced by spermatid quantitation and sperm staging) at exposure levels greater than 1200 ppm in the case of cumene, or greater than 782 ppm (rodents) or 1610 ppm (rabbit) with respect to ethylbenzene. Ovarian toxicity was also assessed in the latter study (and was not demonstrated). These data, coupled with the fact that conventional reproductive toxicity tests in rodents for fertility are an insensitive indicator of reproductive risk in humans (Working, 1988), indicate satisfactory testing. Additionally, testicular histopathological assessments and sperm assessment, which have the highest detection rates for male reproductive effects in animal models (Linder *et al*, 1992; Ulbrich and Palmer, 1995), provide substantial evidence that the reproductive data available for the analogs will suffice to characterize the absence of reproductive effects for the analogs, as well as the diisopropylbenzenes, for which they act as surrogates. It is illogical in my opinion to assume that additional studies beyond what data is provided in the assessment made in this document would be required to establish further the safety shown in the studies evaluated."

"It appears to this reviewer that additional studies on analogs or indeed, the diisopropylbenzene isomers themselves, would only serve the limited objective of confirming the absence of hazard to reproductive and developmental toxicity at reasonable oral or inhalational exposures." (See Appendix I)

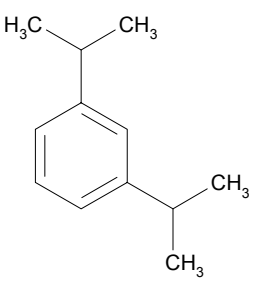
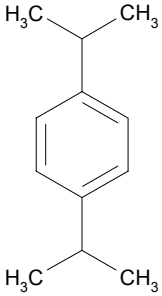
Table 1: Matrix of DIPB and DIPB Surrogates

			DIPB Mixed Isomers	
Common Name	m-Diisopropylbenzene (mDIPB)	p-Diisopropylbenzene (pDIPB)	Diisopropylbenzene (xDIPB)	Cumene (Isopropylbenzene)
CAS No.	99-62-7	100-18-5	25321-09-9	98-82-8
<u>Physico-Chemical</u>				
Melting Point	-61 C	-17.1 C	-40 C	-96 C
Boiling Point	203.2 C	210.3 C	205 C	152.7 C
Density/Sp. G.	0.86	0.86	0.9	0.86
Vapor Pressure	1 mmHg at 34.7 C	1 mmHg at 40 C	0.25-0.39 mmHg 25C	8 mmHg at 20 C
Partition Coeff.	5.40	5.71	4.9	3.55
Water Solubility	7 ppm	3 ppm	1 ppm	50 ppm
Acute Toxicity	>5,000 mg/kg	>5 ml/kg	3,900 mg/kg	2000-4000 mg/kg
Repeat Dose – Target Organs (Oral exposure)	Liver and Kidney	Liver	Liver and Kidney	Liver and Kidney

				
Common Name	Ethylbenzene	o-Diethylbenzene	m-Diethylbenzene	p-Diethylbenzene
CAS No.	100-41-4	135-01-3	141-93-5	105-05-5
<u>Physical-Chemical</u>				
Melting Point	-95 C	-32.2 C	-83.89 C	-42.8 C
Boiling Point	136.25 C	183.4 C	181 C	183.8 C
Density/Sp. G.	0.867	0.88 at 20 C	0.862 at 20 C	0.86
Vapor Pressure	7 mmHg at 20 C	1.1 mmHg at 25 C	1.13 mmHg at 25 C	1.1 mmHg at 25 C
Partition Coeff.	3.13	No Data	4.5	2.87
Water Solubility	152 ppm at 20 C	71 ppm at 25 C	170 ppm	25 ppm
Acute Toxicity	3,900 mg/kg	1,200 mg/kg	1,200 mg/kg	>2000 mg/kg
Repeat Dose – Target Organs	Lung (inhalation exposure), Liver, and Kidney	No Data Available	No Data Available	Liver and Kidney (Oral exposure)

All the above data are representative and were obtained from either Hazardous Substances Database (HSDB), estimation models, or from company MSDS sheets.

II. Matrix of Available Data and Proposed Data Development for Chemicals in the DIPB Category

OECD SIDS Endpoints			DIPB (Mixed isomers)
	m-Diisopropylbenzene	p-Diisopropylbenzene	o-, m-, p-
PHYSICAL-CHEMICAL DATA			
Melting Point	Y[1]	Y	Y
Boiling Point	Y	Y	Y
Vapor Pressure	Y	Y	Y
Partition Coefficient	E[2]	E	E
Water Solubility	Y	Y	E
ENVIRONMENTAL FATE ENDPOINTS			
Photodegradation	E	E	E
Stability in Water	SAR[3]	SAR	SAR
Biodegradation	SAR	Y	Y
Fugacity	E	E	E
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	SAR
Acute Toxicity to Aquatic Invertebrates	Y	Y	SAR
Toxicity to Aquatic Plants	E/SAR	E/SAR	E/SAR
TOXICOLOGICAL DATA			
Acute Toxicity	Y	Y	Y
Repeated Dose Toxicity	Y	SAR	Y
Genetic Toxicity – Mutation	SAR	SAR	Y
Genetic Toxicity – Chromosomal Aberrations	SAR	SAR	Y
Developmental Toxicity	SAR	SAR	SAR
Toxicity to Reproduction	SAR	SAR	SAR
OTHER TOXICITY DATA			
Genetic Toxicity – Primary DNA Damage			Y
Cell transformation Assay			Y

1. Y = Yes, study data specifically on that chemical are available.
2. E = Endpoint was completed through EPA recommended estimation/calculation models.
3. SAR = Endpoint is filled using data from a structurally similar chemical(s).

III. Description of the Test Plan for Each SIDS Endpoint for Each Chemical

Physicochemical Properties

Melting point - **mDIPB -** A value for this endpoint was obtained from reputable textbook.
pDIPB - A value for this endpoint was obtained from reputable textbook.
xDIPB - No value was identified.
Technically data are not needed as these chemicals are liquids with a likely melting points of <0 C.

Boiling Point - **mDIPB -** A value for this endpoint was obtained from reputable textbook.
pDIPB - A value for this endpoint was obtained from reputable textbook.
xDIPB - A value for this endpoint was obtained from reputable textbook.

Vapor Pressure - **mDIPB -** A value for this endpoint was obtained from reputable textbook.
pDIPB - A value for this endpoint was obtained from reputable textbook.
xDIPB - A value for this endpoint was obtained from reputable textbook.

Partition Coefficient - **mDIPB -** A value for this endpoint was obtained from KOWIN, a computer estimation program.
pDIPB - A value for this endpoint was obtained from KOWIN, a computer estimation program.
xDIPB - A value for this endpoint was obtained from KOWIN, a computer estimation program.

Water Solubility - **mDIPB -** A value for this endpoint was obtained by an OECD-TG105 study.
PDIPB - A value for this endpoint was obtained by an experimental study.
xDIPB - A value for this endpoint was obtained from WSKOW v 1.33; a computer estimation program.

Conclusion: **No additional tests are proposed as all end points are satisfied by data from reputable textbooks, actual studies, or acceptable computer modeling estimation programs.**

Environmental Fate

Photodegradation - **mDIPB -** A value for this endpoint was obtained using AOPWIN, a computer estimation program.
pDIPB - A value for this endpoint was obtained using AOPWIN, a computer estimation program.
xDIPB - A value for this endpoint was obtained using AOPWIN, a computer estimation program.

Stability in Water - **mDIPB -** This endpoint is filled with data from an OECD TG-111 study with 1,4 diethylbenzene, a surrogate dialkylbenzene chemical.
pDIPB - This endpoint is filled with data from an OECD TG-111 study with 1,4 diethylbenzene, a surrogate dialkylbenzene chemical.
xDIPB - This endpoint is filled with data from an OECD TG-111 study with 1,4 diethylbenzene, a surrogate dialkylbenzene chemical.

Biodegradation - **mDIPB -** This endpoint was satisfied through the use of data from studies conducted on pDIPB, xDIPB, and 1,4-diethylbenzene.
pDIPB - This endpoint was satisfied through the use of study data on pDIPB and is further supported by data from studies conducted on xDIPB, and 1,4-diethylbenzene.
xDIPB - This endpoint was satisfied through the use of study data on xDIPB and is further supported by data from studies conducted on pDIPB, and 1,4-diethylbenzene.

Fugacity - **mDIPB -** Transport between environmental compartments was determined by using EPIWIN: EQC Level III fugacity computer model.
pDIPB - Transport between environmental compartments was determined by using EPIWIN: EQC Level III fugacity computer model.
xDIPB - Transport between environmental compartments was determined by using EPIWIN: EQC Level III fugacity computer model.

Conclusion: **No additional tests are proposed as all endpoints have been satisfied using data from studies conducted on the various DIPBs, structurally similar compounds, or acceptable computer modeling estimation programs.**

Ecotoxicity Data

Acute Toxicity to Fish - **mDIPB -** This endpoint is filled by data from an OECD TG-203 study.
pDIPB - This endpoint is filled by data from a study that followed a protocol similar to OECD TG-203.
xDIPB - This endpoint is filled by data from mDIPB and pDIPB.

Acute Toxicity to Aquatic Invertebrates - **mDIPB -** This endpoint is filled by data from an OECD TG-202 study.
pDIPB - This endpoint is filled by data from a study that followed a protocol similar to OECD TG-202.
xDIPB - This endpoint is filled by data from mDIPB and pDIPB.

Toxicity to Aquatic Plants - **mDIPB** - This endpoint is filled by data developed by ECOSAR, a computer modeling program, along with data from an OECD TG-201 study on the surrogate chemicals isopropylbenzene and 1,4-diethylbenzene.
pDIPB - This endpoint is filled by data developed by ECOSAR, a computer modeling program, along with data from an OECD TG-201 study on the surrogate chemicals isopropylbenzene and 1,4-diethylbenzene.
xDIPB - This endpoint is filled by data developed by ECOSAR, a computer modeling program, along with data from an OECD TG-201 study on the surrogate chemicals isopropylbenzene and 1,4-diethylbenzene.

Conclusion: **No additional testing is proposed as all endpoints have been satisfied using quality data from studies conducted on the various DIPBs, or through the use of computer modeling in conjunction with actual studies on structurally similar compounds.**

Toxicological Data

Acute Toxicity - **mDIPB -** This endpoint is filled by data from an oral study on mDIPB that followed established protocols under GLP assurances.
pDIPB - This endpoint is filled by data from an oral study on pDIPB that followed established protocols.
xDIPB - This endpoint is filled by data from an oral study on xDIPB that followed established protocols.

Repeat Dose Toxicity -	**mDIPB -** This endpoint is filled with data from an OECD: TG-407 (and Annex V B.7.) 28-Day repeated exposure study conducted on mDIPB under GLP assurances. **pDIPB -** This endpoint is filled with data from a 14-Day repeated exposure study conducted on pDIPB. Target organs identified in this study were similar to ones identified following exposure to mDIPB and xDIPB for 28 days. **xDIPB -** This endpoint is filled with data from a 28-day repeated exposure study conducted on xDIPB that was noted to have followed Japanese guidelines and GLP assurances.
Genetic Toxicity Mutation -	**mDIPB -** This endpoint is filled using surrogate data from two studies conducted on xDIPB under GLP assurances. **pDIPB -** This endpoint is filled using surrogate data from two studies conducted on xDIPB under GLP assurances. **xDIPB -** This endpoint is filled using data from two studies conducted on xDIPB under GLP assurances. One study assessed mutations in *Salmonella typhimurium* and *E. coli* (Ames Assay) and the other evaluated the induction of forward mutations in Chinese hamster ovary cells (CHO/HGPRT). In the Ames assay, xDIPB was noted to be pure mixture. In the CHO/HGPRT study, the chemical utilized was a mixture that historically has contained only 25-40% mixed DIPB isomers.
Aberration -	**mDIPB** - This endpoint is filled using surrogate data from two studies conducted on xDIPB under GLP assurances. **pDIPB** - This endpoint is filled using surrogate data from two studies conducted on xDIPB under GLP assurances. **xDIPB** - This endpoint is filled using data from two studies conducted on xDIPB under GLP assurances. One study was an *in vitro* OECD: TG-473 study, while the other was an *in vivo* mouse micronucleus assay. In the TG-473 study xDIPB was noted to be a pure mixture. In the micronucleus assay, the chemical utilized was a mixture that historically has contained 25-40% mixed DIPB isomers.
Primary DNA Damage -	While not a HPV SIDS endpoint, a robust summary was prepared relative to the potential of a mixture that historically has contained 25-40% mixed DIPB isomers to induce unscheduled DNA synthesis in rat hepatocytes using a protocol identical to an OECD TG-482 study. This study was conducted under GLP assurances.
Developmental and Reproductive Toxicity -	**mDIPB, pDIPB, xDIPB** - This endpoint is filled using surrogate data from studies conducted on various mono- and di-alkyl benzene compounds (isopropylbenzene, ethylbenzene, o-, m-, and p-diethylbenzene). An independent reproductive toxicology consultant validated the scientific suitability for the use of these chemicals and their credibility. His review and assessment can be found in Attachment I. It was his conclusion that additional studies beyond what data are currently available would likely not be useful.
Conclusion:	**No additional testing is proposed as all endpoints have been satisfied with quality data from studies conducted using either one or two of the pure DIPB isomers, on DIPB as a mixed-isomer compound (xDIPB), or from studies on several surrogate chemicals.**

EVALUATION OF DATA FOR QUALITY AND ACCEPTABILITY

The collected data were reviewed for quality and acceptability following the general US EPA guidance (USEPA 1999b) and the systematic approach described by Klimisch *et al.* (1997). These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. This scoring system was only applied to ecotoxicology and human health endpoint studies as recommended by the EPA (USEPA 1999b). The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

(1) Reliable without Restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.

(2) Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.

(3) Not Reliable: Includes studies or data in which there are interferences, or that use non-relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.

(4) Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g., listed in abstracts or secondary literature.

REFERENCES

Bakke, O.M. and Scheline, R.R. (1970). Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol. Appl. Pharmacol.* 16: 691-700.

Bushy Run Research Centre (1989a). Developmental toxicity study of inhaled cumene vapor in CD (Sprague-Dawley) rats. *Project Report 52-621* (Cited by *EINECS Document No. 202-704-5, August, 1999).*

Bushy Run Research Centre (1989b). Developmental toxicity study of inhaled cumene vapor in New Zealand white rabbits. *Project Report 52-622* (Cited by *EINECS Document No. 202-704-5, August, 1999).*

Bushy Run Research Centre (1989c). Cumene (isopropylbenzene) fourteen-week vapor inhalation study in rats with neurotoxicity evaluation. *Project Report 52-628* (Cited by *EINECS Document No. 202-704-5, August, 1999).*

Climie, I. J. G., Hutson, D. H., and Stoydin, G. (1983). The metabolism of ethylbenzene hydroperoxide in the rat. *Xenobiotica* 13:611-618.

Klimisch, H.-J., Andreae, M., and Tillmann, U. (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. *Regul. Toxicol. Pharmacol.* 25:1-5.

Linder, R. E., Strader, L.F., Slott, V.L., and Suarez, J.D. (1992). Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reprod. Toxicol.* 6:491-505.

OECD SIDS Document: Section 3. Data Evaluation, Preparation of SIDS Dossiers and Testing Plans

Saillenfait. A. M., Payan, J. P., Langonne, I., Gallissot, F., Sabate, J. P., Beydon, D., and Fabry, J. P. 91999). Assessment of the developmental toxicity and placental transfer of 1,2-diethylbenzene in rats. *Fd. Chem. Toxicol.* 37: 1089-1096.

Ulbrich, B. and Palmer, A. K. (1995). Detection of effects on male reproduction – literature survey. *J. Am. Coll. Toxicol.* 14: 293-327.

US EPA. 1999a. The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. OPPT, EPA.

USEPA. 1999b. Determining the Adequacy of Existing Data. Guidance for the HPV Challenge Program. Draft dated 2/10/99.

Williams, R. T. (1959). *Detoxication Mechanisms; The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds.* 2nd edition, Wiley, New York.

Working, P.K. (1988). Male reproductive toxicology: comparison of the human to animal models. *Environ. Health Perspect.* 77:37-46.

FINAL REPORT

The Use of Various Mono- and Di-Alkylbenzene Surrogates for the HPV Candidate Diisopropylbenzene Chemicals in SIDS Reproductive/Developmental Toxicity Testing

(WIL-DIPB Literature Review)

Prepared for

American Chemistry Council
Hydroquinone Precursors and Derivatives Panel
(PR'00-075)

Prepared by

James L. Schardein
Consultant in Reproductive and Developmental Toxicology
P.O. Box 37
Chelsea, MI 48118

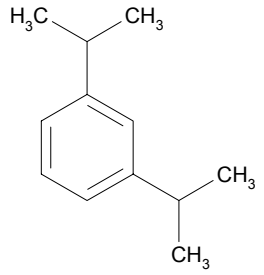
♦ CONTENTS ♦

1. Introduction

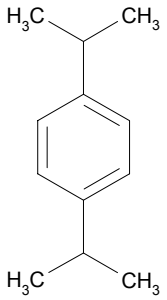
Under the Environmental Protection Agency's (EPA's) High Production Volume (HPV) Chemical Challenge Program, the chemical industry is being challenged to voluntarily compile a Screening Information Data Set (SIDS) for chemicals on the U.S. HPV list. The SIDS, which has been internationally agreed upon by member countries of the Organization for Economic Cooperation and Development (OECD), provides basic screening data needed for initial assessment of the physicochemical properties, environmental fate, and human and environmental effects of chemicals. The information used to complete the SIDS can come from either existing data or from new tests conducted as part of the Challenge Program. In the present case the focus is on fulfilling the developmental (OECD No. 414) and reproductive toxicity testing (OECD Nos. 415 or 422) guidelines.

The Challenge Program chemical list consists of about 2,800 HPV chemicals reported under the Toxic Substance's Control Act's 1990 Inventory Update Rule. The large number of chemicals on the list emphasizes the importance of reducing the number of tests to be conducted, where this is scientifically justifiable.

Pertinent to the present report is the fact that the American Chemistry Council (ACC), through its Hydroquinone Precursors and Derivatives Panel has volunteered various isomers of diisopropylbenzenes under this program. These candidate chemicals include the *m*- and *p*- diisopropylbenzenes (DIPBs) and mixed isomers of diisopropylbenzene:



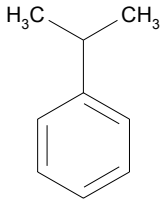
m-diisopropylbenzene



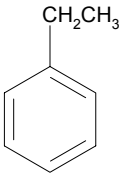
p-diisopropylbenzene

Data available assessing reproductive and developmental toxicity potential on these chemicals are scant and unreliable (see below) (*m*-DIPB) or nonexistent (*p*-DIPB, mixed isomers). As these restrictions most certainly apply to other chemicals in the program as well, EPA has developed a guidance document (EPA, 2000) to assist sponsors in constructing and supporting chemical structure-activity relationships (SAR) for "surrogate" chemicals which might be applied in this program in an effort to reduce the number of tests to be conducted. In the context of this application, SAR is defined as the relationship of the molecular structure of a chemical with a physicochemical property, environmental fate attribute, and/or specific effect on human health or an environmental species to a similar (surrogate) chemical.

The guidance document (EPA, 2000) indicates that SAR may be used in several ways to reduce testing. One of these means is through the identification of a number of structurally similar chemicals as a group or category, and allowing selected members of the group that have been tested, with the results applying to other category members. Accordingly, the Hydroquinone Precursors and Derivatives Panel of ACC has identified the following alkylated benzene compounds as possible surrogate chemicals for the volunteered candidate diisopropylbenzene compounds cited above for the purpose of fulfilling the reproductive/developmental parameters of the SIDS testing. These are:

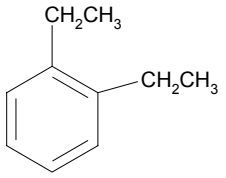


isopropylbenzene
(cumene)



ethylbenzene

o-, m- and *p-* diethylbenzenes



(o-diethylbenzene)

As discussed in the EPA guidance document (2000), these chemicals were chosen as surrogates since they resemble the volunteered candidate in accordance with the specifications for analogs as set forth in that document. In the present case, review of available scientific literature, study adequacy, and possible data gaps have been considered on data from four (4) close analogs of the candidate chemicals with respect to these parameters.

meta-Diisopropylbenzene (CAS 99-62-7), a candidate diisopropylbenzene, was the subject of an earlier report from this reviewer to the ACC Hydroquinone Precursors and Derivatives Panel Report (August 10, 2000). My report concluded that two studies conducted in Russia (some 30 years previously) assessing reproductive toxicity were grossly deficient for regulatory consideration by today's standards in any venue in my opinion. Neither provided "valid core" data, nor were they compliant to Good Laboratory Practice (GLP) standards. Accordingly, these studies would be categorized according to the Klimisch scale as Category 3- "Not Reliable" (Klimisch *et al* 1997), and as such they are not summarized or included in this report (Elisuiskaya, 1970 and Elisuiskaya, 1970).

2. Reproductive/Developmental Toxicity Studies Reported on Surrogate Chemicals

A. Developmental Toxicity Studies

1. Ethylbenzene (CAS 100-41-4)

a. Study 1

There is a developmental toxicity study published on ethylbenzene conducted by the inhalation route at exposure levels of 600, 1200 or 2400 mg/m^3 (recalculated as 138, 276 and 552 ppm) to CFY strain rats, 500 mg/m^3 (115 ppm) to CFLP strain mice and 500 and 1000 mg/m^3 (115 and 230 ppm) to New Zealand breed rabbits (Ungvary and Tatrai, 1985). The rationale for the exposure level selection was not given. Chamber air controls were used in comparison. Exposures were on gd (gestation day) 7-15 for 24 hours per day or gd 18 or 20 for 2-6 hours daily for rats, and on gestation days 6-15 (24 hours/day) for mice and rabbits. By western standards, these exposure periods correspond to one day later (gd 7-15), since positive evidence by harem matings was considered to be day 1 (not gd 0). The exposure intervals comprised primarily the period of major organogenesis in the three species as was the standard procedure at the time (contemporary exposure requirements cover the interval between

implantation (~gd 6) to near-term (~gd 20 for rodents and ~gd 29 or 30 for rabbits). Group sizes ranged from 17-19 in the case of the rat, 20 for mice (one group only), and only 9 for the rabbit (a high dose group of 3 does resulted only in abortion). The current standard group size is approximately 20.

After the exposures were completed in the rats, maternal and fetal blood and amniotic fluid samples were collected to determine the presence of ethylbenzene in those tissue compartments by the use of gas chromatography. It should be mentioned that no detailed description of methodology to evaluate the chemical characteristics of ethylbenezene (e.g., concentration) nor any other analytical parameter of it were in the report, and it is assumed therefore, that none was collected, in contrast to present-day requirements that exist for such characterization. Nonetheless, what was analyzed as described above in blood and amniotic fluid, exceeds the usual procedures.

The animals were euthanized near term as follows: rats, day 21; mice, day 18; rabbits, day 30; the fetuses of all three species were examined by apparently standard methodology which included data on numbers of live, dead and resorbing fetuses; fetal weights; and external, internal and skeletal malformations (both minor and major).

The results of the exposures to rats indicated fetal toxicity at all exposure levels, manifested by marginal but statistically significant ($p<0.05$) increases in death (resorption) and skeletal retardation. At the highest exposure level (552 ppm), there was also marginal (and statistically significant at $p<0.05$) retardation in fetal weight and increased incidence of supernumerary ribs (7 vs. 0% control) and urogenital and skeletal malformations when compared to the control and lower exposure groups (7% vs. 1% control and 3-4% lower exposure groups). The net result was said by the authors to be a mild to moderate teratogenic effect at the highest exposure level of 552 ppm. While the maternally toxic effects of ethylbenzene were described as moderate and dose-dependent, no evidence of that statement was observed in the study results; it was apparent however, that no maternal deaths occurred at any exposure level. Ethylbenezene concentrations were recorded in rat blood and amniotic fluid, and were greater in the former, but both were less in fetal than in maternal tissue compartments. However, no quantitative data were provided on any aspect of ethylbenzene analyses.

The results in mice were somewhat less encompassing than in the rat. However, exposures of 115 ppm were said to result only in a minor increase in urogenital malformations when compared to the controls (10% vs. 4%), but the difference was statistically significant ($p<0.05$). This was termed a mild to moderate teratogenic effect by the authors, but the

specific type of malformations found were not alluded to. The chemical apparently elicited no other developmental (or maternal) toxicity. I believe that the undescribed malformations reported in this species in the absence of other developmental effects to be highly unusual and not convincing of a teratogenic effect.

In the rabbit, exposures of 230 ppm were said to result in mild toxic effects to the mothers, which was manifested by a decrease in maternal weight gain. However, there was abortion in all 3 does exposed to the chemical. At the lower exposure of 115 ppm, the 9 does produced as the sole fetal effect, a statistically significant ($p<0.05$) reduction in mean fetal weight only in the female fetuses; male fetuses had comparable body weights to the air controls. This finding is considered by this reviewer to be insignificant. In contrast to the mouse and rat, no teratogenic effects were reported in the rabbit. Nor was there any reported evidence of maternal toxicity at the lower exposure level of 115 ppm.

In summary, this study was conducted by recognized Hungarian scientists and the results, somewhat scant by western standards, can be taken with some degree of validity, although as I have indicated, questionable conclusions were made in several respects.

b. Study 2

A second developmental toxicity report with ethylbenzene was described in a publication by Hardin *et al* (1981).

In this study, rats (Wistar or Sprague-Dawley strain) in groups of 30 were exposed to ethylbenzene at levels of 0 (filtered air), 100 and 1000 ppm for 7 hours/day, 5 days/week for 3 weeks prior to mating (prebreeding) and/or during gd 1-19. A second species, New Zealand white rabbits, was exposed to 0, 100, 1000 ppm ethylbenzene to groups of 15-20 animals only during gestation (gd 1-24), for 7 hours/day.

The exposure concentrations were selected from published toxicity data and recommended occupational exposure limits. Animals in both studies were euthanized one day prior to term, and maternal and fetal toxicity assessed in the traditional manner.

In the rat study at the exposure level of 1,000 ppm there was maternal toxicity manifested by increased liver, kidney and splenic weights. While there were reduced pregnancy rates at both exposure levels in the prebreeding groups, this effect was not dose-related and thus lacks biological significance. The only fetal effect observed was a significant increase ($p< 0.05$) in extra ribs in offspring of both exposure levels (data not provided). The conclusion was made by the authors of this report that exposure to ethylbenzene at levels above 1,000 ppm may possibly reflect

teratogenic potential. My own interpretation in the absence of actual data is that this conservative view reflects rather only fetotoxicity, in the absence of any other fetal effects, and the designation of possible teratogenesis is unwarranted.

In the rabbit study, there was neither maternal toxicity nor fetal toxicity at either exposure level of 100 or 1,000 ppm ethylbenzene.

While the details were limited in the report reviewed, the results confirm the conclusions in rabbit studies with ethylbenzene by other investigators using the inhalation route of exposure, namely that ethylbenzene is not fetotoxic nor teratogenic in this species.

In the rat, the results confirm the rib anomaly described in Study 1, but do not demonstrate the reported teratogenic effects or other fetotoxicity described in that study.

2. 1,2-Diethylbenzene (o-diethylbenzene, CAS 135-01-3)

This is a developmental toxicity study in rats from a published report by Saillenfait *et al* (1999) conducted with essentially pure 1,2-diethylbenzene (1,2-DEB) by the oral (gavage) route of administration. It is of current scientific standards and is regulatory compliant in all respects.

Doses of 5, 15, 25 or 35 mg/kg of 1,2-DEB were administered by gavage to groups of 28-29 time-mated Sprague-Dawley strain female rats on gd 6-20 (the current OECD/EPA standard). The dosing volume was 2 ml/kg and the controls received corn oil as the (vehicle) control. Dosage levels were determined from previously conducted studies. As in a routine teratology screening study, the dams were euthanized on gd 21, and developmental parameters assessed as follows: numbers of implantations and live/dead fetuses, fetal sex ratios, fetal body weights, and external, visceral and skeletal variations and malformations were determined. The mothers were observed during the experiment for clinical signs of toxicity, and food consumption and body weight were recorded at 3-day intervals. Full statistical analyses were performed.

Placental transfer studies were also performed, on gd 18 at intervals from 1 to 48 hours using a dose level (25 mg/kg) that corresponded to the median maternal and developmental toxic dose in the developmental study described above. In addition, tissue/fluid aliquots were quantitated by liquid scintillation for total radioactivity (^{14}C-label). In still more detailed analyses, known aliquots of plasma, amniotic fluid, and fetal tissue homogenates were extracted and counted for ^{14}C.

The results indicate maternal toxicity at doses of 15, 25 and 35 mg/kg, manifested by statistically ($p<0.05$, 0.01) and biologically significant

reductions in maternal weight gain over the entire treatment period. Weight gain was approximately 50% less than the controls for the high dose (35 mg/kg) group. In addition, maternal food consumption was significantly ($p<0.05$, 0.01) depressed during the initial and final 3 days of treatment at 15 mg/kg and higher on a dose-related basis. These values ranged from approximately 6% of control values at 15 mg/kg, to 10-12% at 35 mg/kg. There were no maternal effects observed at 5 mg/kg.

With respect to developmental toxicity, the only significant parameter was fetal body weight reduction in fetuses at maternal doses of 15 mg/kg and higher; the decreases were dose-related and paralleled the decreased food consumption decreases observed in the mothers. No other developmental toxicity was apparent at any dose level employed. It is clear that 1,2-DEB was not teratogenic in the rat at doses (35 mg/kg) that induced marked maternal toxicity.

Placental transfer studies demonstrated rapid absorption of 1,2-DEB, with all tissues assessed containing radiocarbon within one hour postdose, but placental and fetal tissues accounted for less than 0.35% of the administered dose. Levels of radioactivity in fetuses were lower than those in maternal plasma and placenta at all time points; the highest maternal levels were present in liver and kidney at most all time points. Analyses also indicated that ethyl acetate extractable (acidic) metabolites were predominant in the maternal plasma, while n-hexane extractable (neutral) compounds represented the major part of radioactivity in the placenta and fetus. This suggests poor transfer of 1,2-DEB acidic metabolites to the fetus, and further demonstrates that in the rat at least, the administration of 1,2-DEB and/or metabolites in late gestation results in low exposure levels.

This study was conducted by a well-known and competent investigator and his associates in a recognized French laboratory. The study is considered by this reviewer to be a perfectly acceptable developmental toxicity study carried out for 1,2-diethylbenzene under currently acceptable standards. I concur with the authors' conclusions that this chemical induces developmental toxicity only at marked maternally toxic dose levels in the rat by the oral route. The lack of teratogenic activity under the controlled conditions of the study attest to 1,2-DEB's probable safety.

3. Cumene (1-methylethyl benzene, Isopropylbenzene, CAS 98-82-8)

Two experiments exist on the developmental toxicity potential of cumene. Two species, rat and rabbit, were the test subjects (Darmer, *et al* (1987). The report is abstracted below. The studies were performed according to GLP and to U.S. EPA Guidelines of that time.

In the rat study, groups of 25 CD (Sprague-Dawley) strain female rats were exposed to cumene vapor for 6 hours/day on gd 6-15. Target concentrations of 0 (filtered air), 100, 500 and 1200 ppm were administered by whole body exposures, and were based on results of preliminary range-finding studies. The dams were euthanized on gd 21 and the usual developmental parameters were assessed.

Maternal toxicity was observed in the 1200 ppm group. This toxicity included overt clinical signs (perioral wetness and encrustation), significantly ($p < 0.01$) decreased food consumption during the exposure period, and a 20% reduction in body weight gain ($p < 0.01$) during exposure. Liver weights were also increased relative to body weight ($p < 0.01$). Dams exposed to 500 ppm cumene had a significant ($p < 0.05$) reduction in food consumption, but in the absence of an effect on body weight gain, was considered biologically irrelevant. No findings occurred in dams exposed to cumene levels of 100 ppm. There was no mortality, abortion or early deliveries in any animal at any exposure level.

Developmental parameters in rats were unaffected at all exposure levels. These included viable implantations/litter, sex ratios, fetal body weights, and external, visceral, and skeletal malformations. There were reported significantly *reduced* incidences of dilated ureters and urinary bladder distention in the fetuses of the 1200 ppm group. Decreased incidences of common morphological alterations such as these are not considered toxicologically relevant. None of the selected variations recorded showed increased incidences related to exposures. Cumene did not elicit teratogenicity even at maternally toxic exposures.

In the rabbit study, 15 does per group were exposed to cumene vapor for 6 hours per day on gd 6-18 at concentrations of 0 (filtered air), 500, 1200, and 2300 ppm. As in the rat, exposures were whole-body and were based on preliminary study results. The does were euthanized on gd 29 and full assessment of developmental parameters was made.

Maternal effects consisting of two deaths, one abortion, and significant ($p < 0.01$) reductions in body weight gain and food consumption during the exposure period, clinical signs of toxicity (perioral wetness) both pre- and post-dose, and significant ($p < 0.01$) increase in relative liver weight were observed in the 2300 ppm group. At necropsy, there was discoloration of the lungs in 12% of the does in this group as well. At the lower exposure

levels of 500 and 1200 ppm, reduced food consumption was the only consistent finding, but this was not accompanied by body weight gain inhibition, and thus is not considered a significant biological finding.

With respect to developmental parameters, there were no exposure-related effects observed at any level. These included assessment of numbers of corpora lutea, implantations, live/dead fetuses, sex ratios, pre- and post-implantation losses, fetal body weights, and external, visceral or skeletal variations or malformations.

These study results are sufficiently adequate to deem these studies fully acceptable in characterizing the developmental toxicity of cumene in two species. Scientifically the criteria are met with respect to exposures employed, numbers of animals used, and laboratory characteristics at the time when these studies were done. The only deviation from current protocol apparent in these studies, which was the standard at the time, is that administration of the test article was confined to the period of major organogenesis (rather than from implantation to near-term). In any event, the results clearly demonstrate absence of developmental toxicity, including teratogenicity, at inhalation exposure levels of cumene that induce maternal toxicity.

4. 1,4-Diethylbenzene (p-diethylbenzene, CAS 105-05-5)

This was a study carried out under an OECD combined toxicity and reproductive/developmental toxicity protocol by a contract research facility in 1993. The study was carried out in rats over one generation at doses over the range of 30 to 750 mg/kg. The details of the study are described in full under Section 2.B.1 below. Importantly, the results with respect to developmental toxicity potential clearly demonstrate no developmental toxicity in offspring at oral dose levels up to 750 mg/kg when given to parental animals (Tables 1 and 2).

B. Reproductive Toxicity Studies

1. 1,4-Diethylbenzene (p-diethylbenzene, CAS 105-05-5)

Data are reported on 1,4-diethylbenzene (1,4-DEB) in a study termed an OECD Combined Repeated Dose and Reproductive/Developmental study for High Production Volume Chemicals (presumably OECD No. 422 test guidelines). The study was performed in 1993 by AN-PYO Biosafety Research Center, a contract research facility in Japan. The study described below was abstracted by this reviewer from study outline and tabulated results. It was reported to be subject to 1993 Ministry of Health and Welfare (MHW) requirements in Japan and GLP compliant. However, the abstract displays no test article characterization other than purity, and no statistical analyses were evident.

In this study, 12 slc:SD strain rats of each sex in each group were administered oral (gavage) doses of 30, 150 or 750 mg/kg 1,4-DEB (97.2% purity) prior to mating and subsequently; the males were treated for a total of 44 days including 14 days prior to mating, and the females from 14 days prior to mating, through gestation to postnatal (lactation) day (pd) 3. A control group received a vehicle (unstated) on the same regimen. The study was terminated on pd 3 with euthanization of the dams and pups. It appears that conventional parameters of reproduction and development were assessed.

In the F_0 (parental) animals, there were no overt clinical signs in either sex, nor any mortality at any dose level. Compared to the control and lower dose treated groups, both sexes in the highest dose group (750 mg/kg) exhibited decreased body weights. Food consumption was variable in the male rats, but differences were non-existent in the females. Clinically, there were no hematological effects (males tested only) at any dosage, but males receiving 150 mg/kg had increased levels of BUN and GPT and those (males) treated with 750 mg/kg had increased levels of total protein, albumin, BUN, creatinine, total bilirubin, and GPT, and decreased glucose levels. Again, female rats were not assessed for clinical chemistry parameters. At term, male rats treated with 150 or 750 mg/kg had increased kidney weights (relative and absolute); female rats of either dose level had no similar effect, but rats of both genders receiving 750 mg/kg 1,4-DEB had increased relative and absolute liver weights. Pathologic findings at necropsy were confined to the male rats receiving 750 mg/kg; these findings included liver enlargement with brownish coloration and swelling of the hepatic cells.

Reproductive parameters in the F_0 parental animals did not demonstrate any adverse effects. Fertility rates were 100, 83, 100, and 83% for the control, 30, 150 and 750 mg/kg groups respectively, values showing no test article-related effect. The number of dams with live young paralleled the fertility values. While the duration of pregnancy appeared to be slightly prolonged in the 750 mg/kg group, the difference from the other groups was considered by this reviewer to be within normal expectations. Other parameters, including mean numbers of corpora lutea, implants, live pups at birth and at pd 3, litter weights at birth and at pd 3, and number of abnormal pups were also directly comparable between the treated and control groups. There were somewhat skewed sex ratios of pups in favor of females in the 750 mg/kg group, but this observation has, in my judgement, no biological relevance under these circumstances. While mean individual pup (not litter) weights in the 750 mg/kg group were reduced compared to the controls (5.3 vs. 5.6 g) at birth, this value had reversed by pd 3, where mean weights in the high dose group exceeded the controls (8.5 vs. 8.4 g), thereby negating any interpretation of potential adverse effect.

Based on my interpretation of these results, I place the NOAEL for parental toxicity at 150 mg/kg (decreased body weight at 750 mg/kg), and 750 mg/kg for reproductive and developmental toxicity (no significant effects at 750 mg/kg).

In my opinion, this study performed with 1,4-DEB in the rat according to OECD-type protocol provides a battery of useful information on this chemical. The data presented are consistent with dosages administered, are scientifically acceptable, and appear to be biologically plausible. I accept the conclusion that oral dose levels to male and female rats of 750 mg/kg in this study regimen elicits minor toxicity to parental animals and which does not induce reproductive or developmental toxicity in the resulting offspring.

2. Cumene (1-methylethyl benzene, isopropylbenzene, CAS 98-82-8)

There is no specific reproductive toxicity study on cumene available for review, but a study originally designed as a neurotoxicity study contains data useful in characterizing reproductive aspects of cumene exposure (Cushman *et al* 1995). The report is abstracted below. The study was performed according to GLP and to U.S. EPA Guidelines.

In this study, groups of 15 to 21 male and female Fischer 344 strain rats, were exposed in two subparts to cumene vapor at 0, 50, 100, 500, and 1200 ppm, exposure levels in the range of developmental toxicity studies conducted in rats and rabbits by the same laboratory and considered in this document. The exposures were whole-body, for 6 hours per day, 5 days per week, for 13 weeks. A recovery period of four weeks was present in one subpart. At termination, reproductive organs from male rats of the high exposure (1200 ppm) group and control group were fixed, embedded and stained for histological evaluation by light microscopy. Stages of spermatogenesis were evaluated from the right testis and the left testis was frozen and then homogenized for spermatid counting. Sperm count and sperm morphology were also evaluated. The ovaries of the female rats were weighed.

Microscopically, there were no changes in the male reproductive organs compared to the controls. Further, there were no significant effects of cumene exposure on either quantitative or morphological evaluations of spermatogenesis and no effect on testicular or ovarian weights.

As the reproductive organ parameters examined in males are considered to be representative of testicular toxicity, the results indicate that exposures as high as 1200 ppm to rats do not demonstrate that cumene is toxic to reproduction. Nor do normal ovarian weights suggest toxicity to female rats. I concur with the investigators' conclusions in these regards.

3. Ethylbenzene (CAS 100-41-4)

a. Study 1

No conventional reproductive toxicity study apparently exists for ethylbenzene in laboratory animals. The chemical has been studied by the inhalation route in two subchronic toxicity studies that provide meaningful toxicity data as it relates to reproductive effects. The first was a published study by Cragg *et al* (1989). The study was conducted under standard operating conditions for the timeframe, and would appear to satisfy regulatory requirements in any venue.

In this study, B6C3F1 strain mice and Fischer 344 strain rats were exposed in groups of 20 animals per sex per group to concentrations of essentially pure ethylbenzene of 99, 382 or 782 ppm for 6 hours/day, 5 days/week for 4 weeks (total of 20 whole-body exposures). New Zealand white rabbits were exposed to ethylbenzene at concentrations of 382, 782 or 1610 ppm, also for 6 hours/day, 5 days/week for 4 weeks. Pertinent data, as it relates to reproduction, is that no testicular or ovarian gross or histopathological abnormalities were reported in any of the 3 species when exposed to ethylbenzene at high levels under the study conditions.

b. Study 2

A second subchronic study on ethylbenzene having evaluated reproductive parameters was reported by the National Toxicology Program (Chan, 1992).

In this study, F344/N strain rats and $B6C3F_1$ strain mice of both sexes (group size not provided) were exposed to ethylbenzene vapor (whole body) of 0, 100, 250, 750 or 1,000 ppm for 6 hours/day, 5 days/week for 13 weeks. Among the parameters evaluated with respect to pertinence to reproduction, were sperm examination (motility, concentration of sperm, spermatid head counts) in males and vaginal cytology (estrous cycling) in females. No changes were observed in either evaluation.

I make no claim that these results provide significant data for interpretation of full reproductive toxicity potential. However, the three species study provides indirect and reassuring findings that ethylbenzene is not a reproductive toxicant in either male or female animals.

3. Summary of Reproductive/Developmental Toxicity Existing Studies: Results, Adequacy, and Data Gaps

A. Developmental Toxicity Studies

Studies addressing developmental toxicity have been reported for four (4) analog chemicals. The results of these studies are given in Table 1.

With ethylbenzene, inhalation studies in three species (mouse, rat, rabbit) at levels producing effects demonstrate possible teratogenicity induced in both

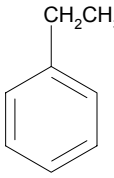
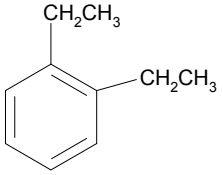
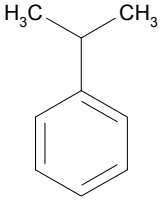
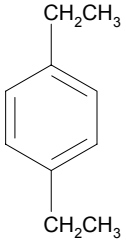
rodent species; there was no teratogenicity produced in the rabbit. The mouse was the most sensitive species, showing equivocal effects (undescribed malformations) at an exposure level of 115 ppm. While these experiments were conducted 15 years ago and lacked a number of currently advocated protocol features, the fact that developmental effects were elicited equivocally is evidence that the effects represent one aspect of the developmental toxicity profile of this analog. Further, the results of these experiments were confirmed in part independently in another laboratory (fetotoxic in rat, not fetotoxic or teratogenic in rabbit), even though there was relatively poor correlation with respect to effect levels in the two studies. Taken together, the two studies characterize, if somewhat imperfectly, the developmental toxicity of ethylbenzene, and constitute in my judgement, sufficient measure of testing adequacy for this chemical (Table 2).

The reported study with a second analog, 1,2-diethylbenzene (1,2-DEB) satisfies all measures of study adequacy as defined by an EPA guidance document (1999). The study is comtemporaneous, was conducted according to present day protocol, and the dose level selection (oral route) resulted in definable levels of developmental toxicity for the species studied (rat). The results indicate a dose-response for both maternal and fetal effects; the chemical is embryotoxic but not teratogenic, at maternally toxic dose levels (Table 2). Biochemistry results indicate poor transfer of 1,2-DEB metabolites to the fetus by this route.

The third analog for which developmental toxicity studies exist is cumene. This study was conducted by a contract laboratory under GLP and protocol details acceptable at the time (1989), and not widely different from present-day standards. Thus, the study fulfills in my opinion, a robust evaluation of cumene exposure in two species with respect to developmental toxicity potential. The results indicate no significant developmental effects, including teratogenicity, induced in either rats or rabbits even at high, maternally toxic exposures to the mothers (Table 2).

The fourth and final study for developmental toxicity potential by diisopropylbenzene analogs is with 1,4-diethylbenzene. This was done by a contract research facility using a combined SIDS study assessing general toxicity and developmental and reproductive effects from a protocol better suited to assess general effects and reproductive effects in a one-generation scenario. However, standard developmental assessments were conducted on all parameters, and the resulting data thus serves to provide meaningful data on 1, 4-DEB. The results demonstrate no significant developmental toxicity to F_1 offspring from oral gavage treatment of parental rats receiving doses of up to 750 mg/kg.

Table 1 Developmental Effects with Diisopropylbenzene Analogs

Chemical	Chemical Structure	CAS No.	Effects Reported	Ref.
Ethylbenzene		100-41-4	*Rat:* -fetotoxic at 138 ppm and higher; possibly teratogenic at 552 ppm -fetotoxic at 100 and 1000 ppm *Mouse:* -questionably teratogenic at 115 ppm *Rabbit:* -abortion at 230 ppm; no fetotoxicity or teratogenicity at 115 ppm -no maternal or developmental toxicity at 1000 ppm	1 2 1 1 2
1,2-Diethylbenzene		135-01-3	*Rat:* -maternal toxicity at 15 mg/kg and higher; developmental toxicity at 15 mg/kg and higher, but not teratogenic	3
Cumene		98-82-8	*Rat:* -maternal toxicity at 1200 ppm; no developmental toxicity including teratogenicity at 1200 ppm *Rabbit:* -maternal toxicity at 2300 ppm; no developmental toxicity including teratogenicity, at 2300 ppm	4 4
1,4-Diethylbenzene		105-05-5	*Rat*: -none at 750 mg/kg	5

1 Ungvary and Tatrai, 1985
2 Hardin *et al* 1981
3 Saillenfait *et al* 1999
4 Darmer *et al* 1997
5 AN-PYO Laboratories, 1993

Table 2 Acceptable Study Characteristics of Developmental Toxicity Studies on Diisopropylbenzene Analogs

Chemical	Species	Route	Dose Range	Developmental NOAEL	Comments	Ref.
Ethylbenzene	CFY rat	I	138-552 ppm	<138 ppm	-fetotoxic at 138 ppm and higher; possibly teratogenic at 552 & 1000 ppm	1
	Wistar or S-D rat	I	100 -1000 ppm	>100 ppm	-fetotoxicity at 1000 ppm	2
	CFLP mouse	I	115 ppm only	none established	questionably teratogenic at 115 ppm	1
	NZ rabbit	I	115-1000 ppm	> 1000 ppm		1,2
1,2-Diethylbenzene	S-D rat	O	5-35 mg/kg	5 mg/kg	-fetotoxicity at 15 mg/kg and higher	3
Cumene	S-D rat	I	100-1200 ppm	> 1200 ppm		4
	NZ rabbit	I	500-2300 ppm	>2300 ppm		4
1,4-Diethylbenzene	S-D rat	O	30-750 mg/kg	>750 mg/kg	parental toxicity at 750 mg/kg	5

I = inhalation O = oral (gavage)

1. Ungvary and Tatrai, 1985
2. Hardin *et al* 1981
3. Saillenfait *et al* 1999
4. Darmer *et al* 1997
5. AN-PYO Laboratories, 1993

B. Reproductive Toxicity Studies

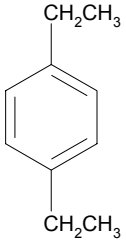
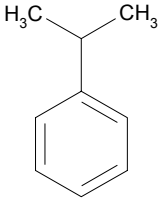
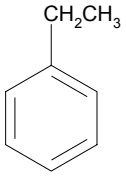
There have been reproductive toxicity studies available for three (3) analogs. The results of these studies are tabulated in Table 3.

The study reported for 1,4-diethylbenzene (1,4-DEB) was done by a contract laboratory using a combined SIDS repeated oral dose and reproductive/developmental toxicity study in 1993, thus the study protocol comprised a late draft version of the OECD No. 422 test guidelines document drafted in 1996. The results indicated parental toxicity at the highest dose tested (750 mg/kg), and no significant reproductive or developmental effects at any level. I accept the conclusion made by the study authors' (Table 4).

The study reported on cumene was conducted by a contract laboratory, and was done to characterize mainly the chronic and neurotoxicity potential of the analog. Carried out under GLP and scientifically acceptable study requirements, the study offered useful reproductive data as it relates to testicular toxicity *per se*; no conventional reproductive parameters were assessed, and thus the study does not serve to be representative of reproductive toxicity potential, only that cumene evidenced no testicular toxicity including sperm evaluation, at exposures as high as 1200 ppm under the conditions of the study. Limited toxicity information in female rats (ovarian weight) also did not suggest adverse effect. It should be stated however, that the study does satisfy an alternative study type which emphasizes reproductive organ assessment (in the male gender) following 90 days exposure (no OECD number for this test) that has been considered an acceptable alternative (Table 4).

Similarly, the studies reported with ethylbenzene on three different species (mouse, rat, rabbit) by the inhalation route is not a conventional reproduction study. Conducted under fairly recent testing guidelines, the studies do not characterize ethylbenzene's reproductive toxicity but does provide convincing evidence that ethylbenzene does not induce testicular or ovarian pathology at exposure levels of 782 ppm (rodents) or 1610 ppm (rabbits), or vaginal cytological or sperm effects in rodents exposed to 1000 ppm (Table 4).

Table 3 Reproductive Effects with Diisopropylbenzene Analogs

Chemical	Chemical Structure	CAS No.	Effects Reported	Ref.
1,4-Diethylbenzene		105-05-5	*Rat:* -none at 750 mg/kg	1
Cumene		98-82-8	*Rat:* -no evidence of gonadal toxicity at 1200 ppm	2
Ethylbenzene		100-41-4	Mouse*:* -no testicular or ovarian pathology at 782 ppm -no sperm or vaginal cytology effects at 1000 ppm *Rat*: -no testicular or ovarian pathology at 782 ppm -no sperm or vaginal cytology effects at 1000 ppm *Rabbit:* -no testicular pathology at 1610 ppm	3 4 3 4 3

1. AN-PYO Laboratories, 1993
2. Cushman *et al* 1995
3. Cragg *et al* 1989
4. Chan, 1992

Table 4 Acceptable Study Characteristics of Reproductive Toxicity Studies on Diisopropylbenzene Analogs

Chemical	Species	Route	Dose Range	Reproductive NOAEL	Comments	Ref.
1,4-Diethylbenzene	S-D rat	O	30-750 mg/kg	>750 mg/kg		1
Cumene	Fischer 344 rat	I	50-1200 ppm	>1200 ppm	no demonstratable effects on male or female reproductive organs or sper-matogenesis	2
Ethylbenzene	B6C3F1 mouse	I	99-782 ppm	>782 ppm	no effects on male or female reproductive organs	3
	Fischer 344 rat	I	99-1000 ppm	>1000 ppm	no effects on male or female reproductive organs	3, 4
	NZ rabbit	I	382-1610 ppm	>1610 ppm	no effects on male or female reproductive organs	3

O = oral (gavage), I = inhalation

1 AN-PYO Laboratories, 1993
2 Cushman *et al* 1995
3 Cragg *et al* 1989
4 Chan, 1992

4. Ancillary Information Supporting Use of Mono- and Dialkylbenzene Surrogates: Metabolism, SAR

Metabolic data on the surrogate chemicals selected demonstrate similar characteristics to the candidate diisopropylbenzene chemicals. While certain polysubstituted methyl derivatives of benzene (e.g., xylenes) undergo ring hydroxylation to form toxic phenols (Gerarde, 1960), others, like diisopropylbenzene, undergo oxidative reactions on their sidechains, with subsequent glucuronidation, not dealkylation, to benzene or phenols. In these cases, the metabolic products are alcohols and/or carboxylic acids which are eventually eliminated in the urine as conjugates of glucuronic acid or glycine (Williams, 1959). This metabolic pathway has been shown for several of the surrogate analogs in the present report, including ethylbenzene (Gerarde and Ahlstrom, 1966; Bakke and Scheline, 1970) and cumene (Robinson *et al* 1955). Further, the toxicological effects produced by the surrogates (i.e., on liver and kidney) are similar to those induced by diisopropylbenzenes.

The SAR specifications promulgated for use of surrogate chemical analogs by EPA (2000) in place of candidate chemicals include comparisons that demonstrate similarity of molecular structure (they are short-chain alkyl derivatives and differ only in substitution position on the benzene ring in the present cases), the analogs belong to a series of well-studied chemicals (alkylbenzenes in these cases), and/or have a similar precursor, metabolite or breakdown product (identical metabolic pathway and metabolic products as described above). In this regard, the focus is on the data available for the analogs and study adequacy.

The correlations to be used with the candidate chemical and the analogs are qualitative predictions based on a comparison of valid measured data from one or more structurally similar compounds (the alkylbenzene analogs cited above) with the candidate chemicals (diisopropylbenzene isomers). Having multiple chemicals in a category, as in the case here, means that experimental data are available for two or more category members, allowing for an analysis that can be extrapolated to other category members with a certain level of confidence. It is recognized (in agreement with the EPA document, 2000) that SAR estimations for health endpoints (reproductive/developmental parameters in this case) must be accompanied by experimental data with a close analog, as already mentioned. In this report, we have made comparisons with several acceptable analogs both with respect to developmental and reproductive toxicity.

5. Conclusions

In summary, the metabolic data assessed demonstrate that at least for ethylbenzene and cumene (and presumably other similar alkylated analogs of the ethylbenzene group), the analog chemicals selected to serve as surrogates for the diisopropylbenzenes, are appropriate from this aspect. The primary route of metabolism is through oxidation and conjugation of the alkyl side chains to chemicals not known to possess significant toxic potential. Further, the physical chemical properties and target organ toxicity are similar to the diisopropylbenzenes.

Similarly, the conditions put forth by EPA on structure-activity relationship (SAR) requirements for selection of surrogate chemicals as discussed above also point to the acceptability of the selected analogs in their use as surrogates to the diisopropylbenzene isomers. I consider the chemicals selected to serve as surrogates to be a valid approach in fulfilling the reproductive/developmental endpoint evaluation for the diisopropylbenzenes, since acceptable data exists on these chemicals (see following).

The existent *developmental toxicity* studies in one (oral route) and three (inhalation route) species and three rodent strains (rat) with the alkylbenzene analogs demonstrate quite convincingly the potential for developmental toxicity in laboratory species. The SIDS requirement is, in fact, for one species testing. In my judgment, no further developmental toxicity studies on the candidate diisopropylbenzene chemicals are needed, as the data on the surrogates suffice. The present data available for interpretation are fully adequate; no data gaps are evident, and additional studies would add little to the database already gleaned from the completed studies with respect to effects on development, by either route of exposure, oral or inhalation. The more critically conducted and robust studies evaluated (Darmer *et al* 1997; Saillenfait *et al* 1999) on the 1,2-DEB and cumene analogs indicate minor embryotoxic or no effects at all at maternally toxic levels, and no teratogenicity.

The results with the *reproductive toxicity* studies conducted on the diisopropylbenzene analogs are less perfect. In fact, only the data from the study conducted on 1,4-DEB is suitable for full characterization of the *conventional* reproductive toxicity assessment of diisopropylbenzene analogs. The remaining three studies, conducted on cumene and ethylbenzene, were not conceived with the objective of fully characterizing their reproductive toxicity potential. However, it cannot be stressed too emphatically, that the studies on the latter two analogs provide much valuable information on the reproductive process in other ways. In both the cumene 90-day inhalation toxicity study in rats and in the ethylbenzene 28 day inhalation toxicity study in three species (see Table 4), alternative study designs that have been considered in the past as acceptable in the SIDS testing scheme, are more than adequate, since there was assessment of the reproductive organs (without mating trial). No toxicity was reported in either study with respect to histopathology of the testes, testicular weight, or the process of spermatogenesis (as evidenced by spermatid quantitation and sperm staging) at exposure levels greater than 1200 ppm in the case of cumene, or greater than 782 ppm (rodents) or 1610 ppm (rabbit) with respect to ethylbenzene. Ovarian toxicity was also assessed in several studies (and was not demonstrated). These data, coupled with the fact that conventional reproductive toxicity tests in rodents for fertility are an insensitive indicator of reproductive risk in humans (Working, 1988) indicate satisfactory testing. Additionally, testicular histopathological assessments and sperm assessment, which have the highest detection rates for male reproductive effects in animal models (Linder *et al* 1992; Ulbrich and Palmer, 1995), provide substantial evidence that the reproductive data available for the analogs will suffice to

characterize the absence of reproductive effects for the analogs, as well as the diisopropylbenzenes, for which they act as surrogates. It is illogical in my opinion to assume that additional studies beyond what data is provided in the assessment made in this document would be required to establish further the safety shown in the studies evaluated.

It appears to this reviewer that additional studies on analogs or indeed, the diisopropylbenzene isomers themselves, would only serve the limited objective of confirming the absence of hazard to reproductive and developmental toxicity at reasonable oral or inhalational exposure levels.

6. References

AN-PYO Biosafety Research Center (1993). [Reproductive/developmental report on 1,4-diethylbenzene in rats]. (Cited in *SIDS Dossier, 1994)*.

Bakke, O.M. and Scheline, R.R. (1970). Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol. Appl. Pharmacol.* 16: 691-700.

Chan, P. (NTP Tox 10) (1992). National Toxicology Program: Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and $B6C3F_1$ mice (inhalation studies). NIH Publication No. 92-3129 (Abstract).

Cragg, S. T., Clarke, E. A., Daly, I. W. *et al* (1989). Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fundam. Appl. Toxicol.* 13:399-408.

Cushman, J. R., Norris, J. C., Dodd, D. E., Darmer, K.I., and Morris, C. R. (1995). Subchronic inhalation toxicity and neurotoxicity assessment of cumene in Fischer 344 rats. *J. Am. Coll. Toxicol*., 14:129-147.

Darmer, K.I., Neeper-Bradley, T. L., Cushman, J. R., Morris, C. R., and Francis, B. O. (1997). Developmental toxicity of cumene vapor in CD rats and New Zealand white rabbits. *Int. J. Toxicol*., 16:119-139.

Elisuiskaya, R.B. (1970). The state of fertility in animals under chronic action of metadiisopropylbenzene vapor. *Medi K T L Sina Truda J Promyshlenna KIL Agekolgi KILA* 14:44-46.

Elisuiskaya, R. V. (1970). The course and outcome of pregnancies in animals exposed to meta-diisopropylbenzene (m-DIPB) vapor. *Gig. Tr. Prof. Zabol.* 4:145.

Environmental Protection Agency (EPA) (1999). Draft guidance on developing robust summaries. Chemical Right-to-Know Initiative, U.S. EPA. Office of Pollution Prevention and Toxics.

Environmental Protection Agency (EPA) (2000). The use of structure-activity relationships (SAR) in the High Production Volume Chemicals Challenge Program. Chemical Right-to-Know Initiative, U.S. EPA, Office of Pollution Prevention and Toxics.

Gerarde, H. W. (1960). *The Toxicology and Biochemistry of the Aromatic Hydrocarbons.* Elsevier, Amsterdam.

Gerarde, H. W. and Ahlstrom, D. B. (1966). Toxicologic studies on hydrocarbons. XI. Influence of dose on the metabolism of mono-n-alkyl derivatives of benzene. *Toxicol. Appl. Pharmacol.* 9: 185-190.

Hardin, B. D., Bond, G. P., Sikov, M. R., Andrew, F. D., Beliles, R. P., and Niemeier, R. W. (1981). Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work Environ. Health*, Suppl. 4:66-75.

Klimisch, H.J., Andreae, M., and Tillmann U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Reg. Toxicol. Pharmacol.* 25:1-5.

Linder, R. E., Strader, L.F., Slott, V.L., and Suarez, J.D. (1992). Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reprod. Toxicol.* 6:491-505.

Robinson, D., Smith, J. N., and Williams, R. T. (1955). Studies in detoxication. 60. The metabolism of alkylbenzenes. Isopropylbenzene (cumene) and derivatives of hydratropic acid. *Biochem. J.* 59: 153-159.

Saillenfait. A. M., Payan, J. P., Langonne, I., Gallissot, F., Sabate, J. P., Beydon, D., and Fabry, J. P. 91999). Assessment of the developmental toxicity and placental trasnfer of 1,2-diethylbenzene in rats. *Fd. Chem. Toxicol.* 37: 1089-1096.

Ulbrich, B. and Palmer, A. K. (1995). Detection of effects on male reproduction – literature survey. *J. Am. Coll. Toxicol.* 14: 293-327.

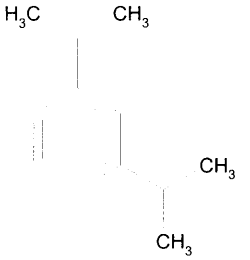
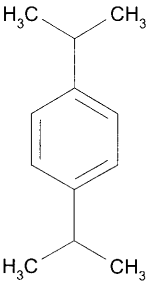
Ungvary, G. and Tatrai, R. (1985). On the embryotoxic effects of benzene and the alkyl derivatives in mice, rats and rabbits. *Arch. Toxicol.* Suppl. 8:425-430.

Williams, R. T. (1959). *Detoxication Mechanisms; The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds.* 2nd edition, Wiley, New York.

Working, P.K. (1988). Male reproductive toxicology: comparison of the human to animal models. *Environ. Health Perspect.* 77:37-46.

ROBUST SUMMARIES

I. General Information

CAS Number:	99-62-7	100-18-5	25321-09-9
Name:	m-Diisopropylbenzene 1,3- Diisopropylbenzene Benzene, 1,3-bis(1-methylethyl)-	p-Diisopropylbenzene 1,4- Diisopropylbenzene Benzene, 1,4-bis(1-methylethyl)-	Diisopropylbenzene Benzene, bis(1-methylethyl) Benzene, diisopropyl- Bis(isopropyl)benzene
Structure:			Material is a variable composition of ortho, meta, and para isomers.

II. Physical-Chemical Data

A. Melting Point

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Melting point value:	-61 ^{o}C
Remarks:	
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5325
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 19980603

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Melting point value:	-17.1 ^{o}C
Remarks:	
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5331
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 980603

B. Boiling Point

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Boiling point value:	203.2 ^{o}C
Pressure:	760 mmHg
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5325
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 19980603

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Boiling point value:	210.3 ^{o}C
Pressure:	760 mmHg
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5331
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 980603

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	Not specified
Method:	Unknown
GLP:	Unknown
Year:	
Remarks:	
Results	
Boiling point value:	205 ^{o}C
Pressure:	Not noted
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 6500
References	National Fire Protection Guide. Fire Protection Guide on Hazardous Materials. Tenth edition, Quincy, MA. National Fire Protection Association, 1991.
Other	Last revision date: 19990921

C. Vapor Pressure

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Vapor pressure value:	1 mmHg
Temperature:	34.7 ^{o}C
Remarks:	
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5325
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 19980603

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Vapor pressure value:	1 mmHg
Temperature:	40.0 ^{o}C
Remarks:	
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 5331
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 980603

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Vapor pressure value:	0.25 – 0.39 mmHg
Temperature:	25 ^{o}C
Remarks:	
Conclusions	
Data Quality	
Remarks:	Data obtained from Hazardous Substances Data Bank Number: 6500
References	Lide, D.R. (Ed.). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press Inc.
Other	Last revision date: 19990921

D. Partition Coefficient

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Log P_{OW}:	4.90
Remarks:	
Data Quality	
Remarks:	
References	KOWIN v1.63; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Log P_{OW}:	3.45
Remarks:	
Data Quality	
Remarks:	
References	KOWIN v1.63; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Estimation
Remarks:	
Results	
Log P_{OW}:	
Remarks:	4.90
Data Quality	
Remarks:	
References	KOWIN v1.63; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

E. Water Solubility

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity was 95.2%
Method	
Method:	OECD: TG-105
GLP:	Yes
Year:	1986
Remarks:	
Results	
Value:	7.01 mg/L (7 ppm)
Temperature:	Initial water bath on days 4-7 was 30 ^{o}C; this was followed by a one-day re-equilibration period with a temperature of 25 ^{o}C.
Description:	Negligible
Remarks:	
Data Quality	
Remarks:	Study was an OECD guideline study conducted by the Chemicals Quality Services Division, at Eastman Kodak Company, Rochester, NY.
References	Final report: Water Solubility (Attachment 3) in Acute Aquatic Effects of m-Diisopropylbenzene on Seven Freshwater Species HAEL: 85-0077, August 19, 1986.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other: Precipitation-Nephelometric
GLP:	No
Year:	1984
Remarks:	
Results	
Value:	3.0 mg/L (3 ppm)
Temperature:	Not noted in report
Description:	Negligible
Remarks:	
Data Quality	
Remarks:	
References	Basic Environmental Profile for: p-Diisopropylbenzene; Chemicals Quality Services Division, Eastman Kodak Company, Rochester, NY; HAEL: 82-0014, February 9, 1984.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Estimation
Remarks:	
Results	
Value:	4.325 mg/L
Temperature:	25 ^{o}C
Description:	A log Kow of 4.91 was used in the estimation
Remarks:	
Data Quality	
Remarks:	
References	WSKOW v1.33; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

II. Environmental Fate Endpoints

A. Photodegradation

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Test type:	Atmospheric oxidation
Remarks:	
Results	
Temperature:	25 ^{o}C
Hydroxyl radicals reaction	
OH Rate constant:	15.5240 x 10^{-12} cm^3/molecule-sec
Half-life:	0.689 Days (12-hr day; 1.5x10^6 OH/cm^3)
Ozone reaction:	No ozone reaction estimation
Remarks:	
Conclusions	Material readily reacts with atmospheric hydroxyl radicals.
Data Quality	
Remarks:	
References	AopWin v1.88; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Test type:	Atmospheric oxidation
Remarks:	
Results	
Temperature:	25 ^{o}C
Hydroxyl radicals reaction	
OH Rate constant:	10.1158 x 10^{-12} cm^3/molecule-sec
Half-life:	1.057 Days (12-hr day; 1.5x10^6 OH/cm^3)
Ozone reaction:	No ozone reaction estimation
Remarks:	
Conclusions	Material readily reacts with atmospheric hydroxyl radicals.
Data Quality	
Remarks:	
References	AopWin v1.88; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Estimation
Test type:	Atmospheric oxidation
Remarks:	
Results	
Temperature:	25 ^{o}C
Hydroxyl radicals reaction	
OH Rate constant:	10.1158 x 10^{-12} cm^3/molecule-sec
Half-life:	1.057 Days (12-hr day; 1.5x10^6 OH/cm^3)
Ozone reaction:	No ozone reaction estimation
Remarks:	
Conclusions	Material readily reacts with atmospheric hydroxyl radicals.
Data Quality	
Remarks:	
References	AopWin v1.88; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

B. Stability in Water

Test Substance	
Test substance:	1,4-Diethylbenzene
Remarks:	Purity unknown
Method	
Method:	OECD TG-111
Test type:	Abiotic hydrolysis
GLP:	Yes
Year:	1993
Remarks:	Assessments were made at pH 4, 7, and 9.
Results	
Degradation %:	No hydrolysis was noted at any of the three levels of pH tested.
Remarks:	
Conclusions	
Data Quality	
Remarks:	This study was presented in the OECD SIDS dossier for this chemical.
References	MITI, Japan (1993)
Other	

C. Biodegradation

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other
Test type:	21-Day biodegradation
GLP:	No
Year:	1984
Contact time:	21-Days
Inoculum:	An acclimated culture of microorganisms
Remarks:	There was essentially no detail present in the report regarding methodology.
Results	
Degradation %:	0%
Results:	No degradation was noted based on a lack of CO_2 evolution
Remarks:	These results are in alignment with what was observed with another p-dialkylated-benzene (1,4-diethylbenzene). Using OECD TG 301C, there was no degradation following a 28-day incubation with activated sludge (OECD SIDS dossier on CAS No. 105-05-5; March 1994).
Conclusions	Material does not appear to be readily degraded by microorganisms in an aqueous environment.
Data Quality	
Remarks:	While there was essentially no information in the report relative to the methodology used. It was still assigned this high a level of reliability based on the fact that this study was carried out at a laboratory with an established history of conducting biodegradation studies and the results are similar to those reported using CAS No. 25321-09-9 (mixed isomer of diisopropylbenzene) as well as results observed using another dialkylated benzene molecule benzene (1,4-diethylbenzene).
References	Basic Environmental Profile For: p-Diisopropylbenzene; Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY; HAEL: 82-0014, February 9, 1984.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Other
Test type:	21-Day biodegradation
GLP:	No
Year:	Unknown
Contact time:	21-Days
Inoculum:	Activated sludge
Remarks:	There was essentially no detail present in this report regarding methodology. However it was noted that the test conditions consisted 100 mg/L of test substance, 30 mg/L suspended solid of activated sludge, 25 ^{o}C.
Results	
Degradation %:	BOD 2% and gas chromatography 0%
Results:	Essentially no degradation was noted
Remarks:	These results are in alignment with what was observed with another p-dialkylated-benzene (1,4-diethylbenzene). Using OECD TG 301C, there was no degradation following a 28-day incubation with activated sludge (OECD SIDS dossier on CAS No. 105-05-5; March 1994).
Conclusions	Material does not appear to be readily degraded by microorganisms in an aqueous environment.
Data Quality	
Remarks:	Although there was essentially no information given about the conduct of this study at the website where the data were found, the results are identical to those observed with a pure isomer of this CAS No., p-DIPB, as well as with a structurally related dialkylbenzene, 1,4- diethylbenzene. Consequently, these results are in all likelihood accurate.
References	Chemicals Evaluation and Research Institute, Japan. Internet Web Address: http://www.citi.or.jp/e_index.htm
Other	

D. Transport between Environmental Compartments (Fugacity)

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	
Method	
Test type:	Estimation
Model used:	Level III Fugacity Model; EPIWIN:EQC from Syracuse Research Corporation
Remarks:	
Results	
Model data and results:	Concentration (%)
Estimated distribution and media conc. (levels II/III)	Air 1.73 Water 12.2 Soil 80.4 Sediment 5.66
Remarks:	Physical chemical parameters from the EPIWIN program used to estimate distribution concentrations were: Temperature (25 ^{o}C) water solubility (4.325 mg/L), vapor pressure (0.259 mmHg), Log Kow (4.90), melting point (-16.12 ^{o}C), Henry LC (2.81 x 10^{-2} atm-m^3/mole), and Log Koc (3.606).
Conclusions	
Data Quality	
Remarks:	
References	Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. The Level III model incorporated into EPIWIN is a Syracuse Research Corporation adaptation of the methodology described by Mackay *et al.* 1996; *Environ. Toxicol. Chem.* 15(9), 1618-1626 and *Environ. Toxicol. Chem.* 15(9), 1627-1637.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	
Method	
Test type:	Estimation
Model used:	Level III Fugacity Model; EPIWIN:EQC from Syracuse Research Corporation
Remarks:	
Results	
Model data and results:	Concentration (%)
Estimated distribution and media conc. (levels II/III):	Air 2.39 Water 12.1 Soil 79.9 Sediment 5.62
Remarks:	Physical chemical parameters from the EPIWIN program used to estimate distribution concentrations were: Temperature (25 ^{o}C) water solubility (4.325 mg/L), vapor pressure (0.259 mmHg), Log Kow (4.90), melting point (-16.12 ^{o}C), Henry LC (2.81 x 10^{-2} atm-m^3/mole), and Log Koc (3.606).
Conclusions	
Data Quality	
Remarks:	
References	Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. The Level III model incorporated into EPIWIN is a Syracuse Research Corporation adaptation of the methodology described by Mackay *et al.* 1996; *Environ. Toxicol. Chem.* 15(9), 1618-1626 and *Environ. Toxicol. Chem.* 15(9), 1627-1637.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Test type:	Estimation
Model used:	Level III Fugacity Model; EPIWIN:EQC from Syracuse Research Corporation
Remarks:	
Results	
Model data and results:	Concentration (%)
Estimated distribution and media conc. (levels II/III):	Air 2.39 Water 12.1 Soil 79.9 Sediment 5.62
Remarks:	Physical chemical parameters from the EPIWIN program used to estimate distribution concentrations were: Temperature (25 ^{o}C) water solubility (4.325 mg/L), vapor pressure (0.259 mmHg), Log Kow (4.90), melting point (-16.12 ^{o}C), Henry LC (2.81 x 10^{-2} atm-m^3/mole), and Log Koc (3.615).
Conclusions	
Data Quality	
Remarks:	
References	Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. The Level III model incorporated into EPIWIN is a Syracuse Research Corporation adaptation of the methodology described by Mackay *et al.* 1996; *Environ. Toxicol. Chem.* 15(9), 1618-1626 and *Environ. Toxicol. Chem.* 15(9), 1627-1637.
Other	

III. Ecotoxicity

A. Acute Toxicity to Fish

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity was 95.2%
Method	
Method:	OECD:TG-203
Test type:	Static
GLP:	Yes
Year:	1986
Species/strain:	Fathead minnow (*Pimephales promelas*)
Analytical monitoring:	Temperature, pH, and dissolved oxygen were analyzed at 0, 24, 48, 72, and 96 hours. Test material concentration was assessed at 0, 48, and 96 hours.
Exposure period:	96-Hour
Remarks:	Study was conducted in duplicate using 10 fish per tank with loading kept below 0.5 g/L. The lighting regimen consisted of 16 hours on and 8 hours off with a 20-minute transition period.
Results	
Nominal concentration:	50 mg/L
Measured conc.:	0.91 mg/L (Average of the two samples)
Endpoint value:	LC_{50} >0.91 mg/L; NOEC >0.91 mg/L
Biological obs.:	All fish exhibited normal behavior.
Statistical methods:	NA, There were no deaths noted and only one concentration was tested.
Remarks:	No significant protocol deviations were noted that would affect study results. Mean hardness and total alkalinity were 131 and 93 ppm respectively. The pH ranged from 8.0-8.3, dissolved oxygen was 7.1-8.0 mg/L, and temperature was 20-21 ^{o}C. Test solution was maintained at a saturating level through a recirculating elutriation system.
Conclusions	Material is not toxic to fish at saturating levels.
Data Quality	
Reliability:	1; Reliable without restrictions
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances.
References	Acute Aquatic Effects of m-Diisopropylbenzene on Seven Freshwater Species; Environmental Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY; HAEL: 85-0077, August 19, 1986.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other
Test type:	Static
GLP:	No
Year:	1984
Species/strain:	Fathead minnow (*Pimephales promelas*)
Analytical monitoring:	Not noted in report
Exposure period:	96-Hour
Remarks:	
Results	
Nominal concentration:	3 mg/L (Test solution was believed to be at a saturation level.)
Measured conc.:	Not conducted
Endpoint value:	LC_{50} >3 mg/L; NOEC >3 mg/L
Biological obs.:	All fish exhibited normal behavior.
Statistical methods:	NA, There were no deaths noted and only one concentration was tested.
Remarks:	No significant protocol deviations were noted that would affect study results.
Conclusions	Material is not toxic to fish at saturating levels.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	
References	Basic Environmental Profile for: p-Diisopropylbenzene; Environmental Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY; HAEL: 82-0014, February 9, 1984.
Other	

B. Acute Toxicity to Aquatic Invertebrates

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity was 95.2%
Method	
Method:	OECD: TG-202
Test type:	Acute immobilization
GLP:	Yes
Year:	1986
Species/strain:	Daphnia magna
Analytical monitoring:	Aliquots of exposure solution were submitted for concentration determinations at 0, 24, and 48 hours. Temperature, dissolved oxygen, and pH were also determined at these same time periods.
Test details:	48-hour exposure period; static
Remarks:	No protocol deviations were noted. Study was conducted in duplicate and results were averaged.
Results	
Nominal concentration:	1 mg/L
Measured conc.:	0.93 mg/L (Average of the two samples)
Endpoint value:	LC_{50} >0.93 mg/L; NOEC >0.93 mg/L
Biological obs.:	The *Daphnia* exhibited behavior comparable to controls.
Statistical methods:	NA, There were no effects noted and only one concentration was tested.
Remarks:	No significant protocol deviations were noted that would affect study results.
Conclusions	Material is not toxic to *Daphnia* at near saturating levels.
Data Quality	
Reliability:	Reliable without restrictions
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances.
References	Acute Aquatic Effects of m-Diisopropylbenzene on Seven Freshwater Species; Environmental Sciences Section, Health and Environment Laboratories Eastman Kodak Company, Rochester, NY; HAEL: 85-0077, August 19, 1986.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other
Test type:	Acute immobilization
GLP:	No
Year:	1984
Species/strain:	Daphnia magna
Analytical monitoring:	Not noted in report.
Test details:	No protocol deviations were noted.
Remarks:	
Results	
Nominal concentration:	3 mg/L (Test solution was believed to be at a saturation level.)
Measured conc.:	Not conducted
Endpoint value:	LC_{50} >3 mg/L; NOEC >3 mg/L
Biological obs.:	There was no difference between the responses seen in control or treated
Statistical methods:	Daphnia.
Remarks:	NA, There were no effects noted and only one concentration was tested. No significant protocol deviations were noted that would affect study results.
Conclusions	Material is not toxic to *Daphnia* at saturating levels.
Data Quality	
Reliability:	Reliable with restrictions
Remarks:	
References	Environmental Profile for: p-Diisopropylbenzene; Environmental Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY; HAEL: 82-0014, February 9, 1984.
Other	

C. Toxicity to Aquatic Plants

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Test type:	96-hour
Remarks:	
Results	
EC_{50}:	4.2 mg/L
Remarks:	This estimated value would in all likelihood be very comparable to values obtained through actual testing. This conclusion is based on the results from studies using isopropylbenzene (cumene) and 1,4-diethylbenzene, which are structurally similar to xDIPB. Both studies were noted to have followed standard OECD TG-201 protocols and full summaries of their data should be available through the OECD SIDS program. The estimated and actual EC_{50} vales for isopropylbenzene are 3.1 and 2.6 mg/L, respectively. The respective actual and estimated vales for 1,4-diethylbenzene are 29 and 3.5 mg/L. It is important to note that the "actual" concentration value listed for 1,4-diethylbenzene exceeded the listed water solubility value of 17 mg/L.
Conclusions	
Data Quality	
Reliability:	2, Reliable with restrictions
Remarks:	
References	1.) ECOSAR; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. 2.) OECD SIDS dossiers for Cumene and 1,4-Diethylbenzene.
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	
Method	
Method:	Estimation
Test type:	96-hour Green Algae EC_{50}
Remarks:	
Results	
EC_{50}:	4.2 mg/L
Remarks:	This estimated value would in all likelihood be very comparable to values obtained through actual testing. This conclusion is based on the results from studies using isopropylbenzene (cumene) and 1,4-diethylbenzene, which are structurally similar to xDIPB. Both studies were noted to have followed standard OECD TG-201 protocols and full summaries of their data should be available through the OECD SIDS program. The estimated and actual EC_{50} vales for isopropylbenzene are 3.1 and 2.6 mg/L, respectively. The respective actual and estimated vales for 1,4-diethylbenzene are 29 and 3.5 mg/L. It is important to note that the "actual" concentration value listed for 1,4-diethylbenzene exceeded the listed water solubility value of 17 mg/L.
Conclusions	
Data Quality	
Reliability:	2, Reliable with restrictions
Remarks:	
References	1.) ECOSAR; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. 2.) OECD SIDS dossiers for Cumene and 1,4-Diethylbenzene.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Estimation
Test type:	96-hour Green Algae EC_{50}
Remarks:	
Results	
EC_{50}:	0.219 mg/L
Remarks:	This estimated value would in all likelihood be very comparable to values obtained through actual testing. This conclusion is based on the results from studies using isopropylbenzene (cumene) and 1,4-diethylbenzene, which are structurally similar to xDIPB. Both studies were noted to have followed standard OECD TG-201 protocols and full summaries of their data should be available through the OECD SIDS program. The estimated and actual EC_{50} vales for isopropylbenzene are 3.1 and 2.6 mg/L, respectively. The respective actual and estimated vales for 1,4-diethylbenzene are 29 and 3.5 mg/L. It is important to note that the "actual" concentration value listed for 1,4-diethylbenzene exceeded the listed water solubility value of 17 mg/L.
Conclusions	
Data Quality	
Reliability:	2, Reliable with restrictions
Remarks:	
References	1.) ECOSAR; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210. 2.) OECD SIDS dossiers for Cumene and 1,4-Diethylbenzene.
Other	

IV. Toxicological Data

A. Acute Toxicity

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity was 95.2%
Method	
Method:	Other
Test type:	Acute lethality; LD_{50} estimate
GLP:	Yes
Year:	1985
Species/strain:	Rat/CRL:CD(SD)BR
Sex:	Both
Animals/sex/dose:	5/sex/dose
Vehicle:	None
Route of exposure:	Oral
Remarks:	Animals were administered the test material by oral gavage at a rate of 1250, 2500, or 5000 mg/kg. At the start of the study the males weighed 136-149 g and females were 143-160 g. They were monitored for 14 days after which they were sacrificed and grossly examined.
Results	
Value:	LD_{50} = >5000 mg/kg
Deaths at each dose:	No deaths were noted at any dose.
Remarks:	No behavioral or gross pathological changes were noted. All animals gained weight.
Conclusions	Material is practically non-toxic
Data Quality	
Reliability:	1, Reliable without restrictions
Remarks:	This was a well-documented study conducted under GLP assurances.
References	Acute Toxicity of m-Diisopropylbenzene; 1,3-Diisopropylbenzene; Toxicological Sciences Section, Health and Environment Laboratories Eastman Kodak Company, Rochester, NY.; HAEL No.: 85-0077; December 20, 1985
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other
Test type:	Acute lethality; LD_{50} estimate
GLP:	No
Year:	1982
Species/strain:	Rat
Sex:	Both
Animals/sex/dose:	4/sex/dose
Vehicle:	None
Route of exposure:	Oral
Remarks:	Animals were fasted overnight prior to administration of the test substance. Dose levels were 1600 and 3200 mg/kg. At study start, animals weighed 170 to 226 grams. No necropsies were conducted.
Results	
Value:	LD_{50} = >3200 mg/kg
Deaths at each dose:	No deaths were noted at any dose.
Remarks:	Immediately after dosing all animals appeared slightly weak. Slight to moderate weakness and roughened hair coats were noted at one hour, and slight weakness and rough hair coats were noted at two hours after dosing. By four hours after administration of the test substance, no abnormal clinical signs were observed in either sex at either dose level. No further abnormal clinical signs were observed at any time during the 14-day observation period. All animals gained weight during the study.
Conclusions	Based on the highest does administered the material would be classified as slightly toxic.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	
References	Basic Toxicity of p-Diisopropylbenzene; Toxicological Sciences Section, Health and Environment Laboratories Eastman Kodak Company, Rochester, NY. HAEL No.: 82-0014; April 16, 1984.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Other
Test type:	Acute lethality; LD_{50} estimate
GLP:	No
Year:	1976
Species/strain:	Rat/ Sprague Dawley albino
Sex:	Both
Animals/sex/dose:	5/dose
Vehicle:	None
Route of exposure:	Oral
Remarks:	Groups of 3 males and 2 females, or vice versa, for a total of 5 were administered undiluted test article at a rate of 3160, 3980, 5010, and 6310 mg/kg. Animals were monitored for 14 days, after which they were terminated and examined grossly.
Results	
Value:	LD_{50} = 3900 mg/kg
Deaths at each dose:	3160 (0/5), 3980 (3/5), 5010 (3/5), and 6310 mg/kg (5/5)
Remarks:	Deaths were noted between one and five days with most noted to have occurred within the first 2 days. Surviving animals exhibited reduced appetite and activity during Days 1-3. Gross examination of the animals dying before 14 days showed hemorrhagic areas in the lungs, liver discoloration, and acute gastrointestinal inflammation. Viscera of animals that survived to Day 14 were normal in appearance.
Conclusions	Material would be classified as slightly toxic.
Data Quality	
Reliability:	1; Reliable without restrictions
Remarks:	This is a well-documented study conducted prior to the inaction of GLP requirements.
References	Younger Laboratories Inc., St. Louis, MO Project No. Y-76-347; November 5, 1976.
Other	

B. Repeated Dose Toxicity

Test Substance	
Test substance:	m-Diisopropylbenzene
Remarks:	Purity was 95.2%
Method	
Method:	OECD: TG-407 and Annex V B.7.
Test type:	Repeated exposure
GLP:	Yes
Year:	1986
Species/strain:	Rat/CRL:COB CD(SD)BR
Route of exposure:	Oral gavage
Duration of test:	29 days
Dose levels:	100, 300, and 1000 mg/kg
Sex:	Both, 5/sex/dose
Exposure period:	21 doses
Frequency of treatment:	1x/day
Control group and treatment:	Water gavage
Post-exposure observation period:	None
Remarks:	At the start of study, 7-8 week old rats weighed approximately 223 g (males) and 187 g (females). Body weight and feed consumption were monitored on a weekly basis. Complete clinical examinations were performed once per week and cage-side examination were performed daily. Complete hematology and clinical chemistry examinations were completed at termination. The liver and kidney (previously identified as target organs) were the only organs weighed. Histology was completed on these two organs plus 27-28 additional ones. All gross lesions were excised and examined by microscope.
Results	
NOEL:	Not determined
LOEL:	100 mg/kg
Toxic responses by dose:	100 mg/L – The only effects noted were a gastric irritation (hyperkeratosis) in the non-glandular region of one female and the formation of hyaline droplets in the renal proximal tubules of males. 300 mg/kg – A slight increase in the relative liver weights were seen in both sexes with the change noted as significant in females. Upon microscopic examination one male was noted to have slightly enlarged hepatocytes. Evidence of gastric irritation (hyperkeratosis, edema and focal necrosis) was noted in the non-glandular region of 2/5 males and 1/5 females. Hyaline droplet formation was noted in the renal proximal tubules of males. 1000 mg/kg – Excessive salivation was noted immediately after dosing in one female on Day 27 and in two males on the Day 27 and 28. Males showed a slight decrease in serum glucose and a slight increase in serum creatinine levels. Absolute liver weights were increased in males and relative weights were increased in both sexes. All males showed evidence of hepatocyte hypertrophy. Evidence of gastric irritation characterized by hyperkeratosis (M: 5/5, F: 4/5) and acanthosis (M: 3/5; F: 1/5) was noted in the non-glandular region. Edema was noted in the stomach of 1/5 females too. Hyaline droplet formation was noted in the renal proximal tubules of males.

Statistical methods:	One-way ANOVA, Bartlett's test, and Duncan's multiple range test using a P value of <0.05 to indicate significance.
Remarks:	While a NOEL was not established due to the effects seen in stomachs, it is important to note that this effect was likely a direct irritant response and not the result of systemic toxicity. The only evidence of a systemic effect due to mDIPB was that of mild liver hypertrophy, only manifested at the mid- and high-dose levels. The absolute weight of livers in females was not affected at all and the liver weight to body weight ratios at the 300 mg/kg dose was only 3.3 and at the 1000 mg/kg dose was 3.5 verse a ratio of 3.2 in controls. This effect is often not considered an adverse effective but an adaptive induction of metabolic enzymes subsequent to a repeated exposure to high doses of a chemical. There were no increases noted in serum liver enzymes that would be more indicative of toxicity to the liver. Although the slight increases noted in male serum glucose and creatinine were statistically significant, there biological significance is minimized by the fact that these values were within historical control levels and was only seen in a single sex. The effects noted in the kidneys of males appeared to be an accumulation of hyaline droplets. This effect is unique to male rats following exposure to branched chain compounds and is not believed relevant to humans.
Conclusions	m-Diisopropylbenzene was well tolerated by rodents with the primary effects being an irritation of the stomach and increased liver weights.
Data Quality	
Reliability:	1; Reliable without restrictions
Remarks:	This was an OECD-guideline study conducted under GLP assurances.
References	Four-Week Oral Toxicity of m-Diisopropylbenzene in the Rat; Toxicological Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY; HAEL No.: 85-0077; Experiment No.: 85-0077G2; February 7, 1986
Other	

Test Substance	
Test substance:	p-Diisopropylbenzene
Remarks:	Purity was 99.6%
Method	
Method:	Other
Test type:	Repeated exposure
GLP:	No
Year:	1981
Species/strain:	Rat
Route of exposure:	Oral gavage
Duration of test:	15 days
Dose levels:	100 and 1000 mg/kg
Sex:	Male (5/dose)
Exposure period:	11 doses
Frequency of treatment:	1x/day
Control group and treatment:	Water gavage (1000 mg/kg)
Post-exposure observation period:	None
Remarks:	Body weight and feed consumption were monitored on a weekly basis. Complete clinical examinations were performed once per week and cage-side examination were performed daily. Complete hematology and clinical chemistry examinations were completed at termination. The liver, kidney, and spleen were the only organs weighed. Histology was completed on these organs plus gross lesions.
Results	
NOEL:	100 mg/kg
LOEL:	1000 mg/L
Toxic responses by dose:	100 mg/L – No effects were noted at this dose. 1000 mg/kg – A single animal exhibited a porphyrin nasal discharge and weight loss. It was believed to be due to aspiration pneumonia. A slight but statistically significant increase in platelets and percentage of monocytes were the only changes in hematology. A moderate decrease in glucose and an increase in serum creatinine were noted. The relative liver weight was increased in a statistically significant manner. An enlargement of the heart was noted upon gross examination in 3/5 animals, however, these animals exhibited lung consolidation and this was interpreted as a secondary effect. There were no compound effects noted in any tissue (including the heart) following microscopic examination.
Statistical methods:	Not noted in report
Remarks:	
Conclusions	p-Diisopropylbenzene was well tolerated by rodents with the primary effects being an increase in liver weight. The effect noted in the heart and blood was in all likelihood secondary to gavage error.

Data Quality Reliability: Remarks:	2; Reliable with restrictions
References	Basic Toxicity of p-Diisopropylbenzene; Toxicological Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY; HAEL No.: 82-0014; April 16, 1984.
Other	

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Purity was noted as 99.8%. Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	Guidelines for 28-day Repeat Dose Toxicity Testing of Chemicals (Japan)
Test type:	Repeated exposure
GLP:	Yes
Year:	Unknown
Species/strain:	Rat/Crj:CD(SD)
Route of exposure:	Oral gavage
Duration of test:	29 Days
Dose levels:	6, 30, 150, and 750 mg/kg
Sex:	Both, 6/sex/dose (12 animals were given 0 and 750 mg/kg)
Control group and treatment:	Corn oil
Post-exposure obs. period:	14 days (control and high dose)
Remarks:	
Results	
NOEL:	30 mg/kg
Toxic responses by dose:	6 and 30 mg/kg - No effects were noted. 150 mg/kg - Mydriasis was observed in males and females. 750 mg/kg - Mydriasis was observed in males and females. Blood chemical examinations showed a decrease in chloride in both sexes and an increase in potassium in males. While females exhibited an increase in total protein, total cholesterol and phospholipids. An increase in liver weight was noted in both sexes and kidney weights in males. Histopathological analysis revealed centrilobular hypertrophy of hepatocytes in males and females. Furthermore, the incidence of eosinophilic bodies in proximal tubules of the kidney was increased in males. Following a 14-day recovery period, there were no differences between control and treated groups.
Statistical methods:	Unknown
Remarks:	The effects noted in the kidneys of males were likely due to an accumulation of hyaline droplets.
Conclusions	Diisopropylbenzene was well tolerated by rodents with the primary effect being an increase in liver weight. Animals readily recovered following a 14-day cessation of exposure.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	This summary was obtained from the referenced website and a full report was not available. The summary for this study was relatively scant and it is unknown what year it was conducted, nevertheless, this study was noted to have followed established guidelines under GLP assurances.
References	Safety Assessment Laboratory, Panapharm Laboratories Co., Ltd. 1285 Kurisaki-machi, Uto-shi, Kumamoto, 869-0425, Japan Tel +81-964-23-5111 Fax +81-964-23-2282 (http://wwwdb.mhw.go.jp/ginc/)
Other	

C. Genetic Toxicity – Mutation

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Purity was 99.8%. Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	OECD: TG-471 and 472
Test type:	*In vitro* mutagenicity, pre-incubation method
GLP:	Yes
Year:	Unknown
Species/strain:	*Salmonella typhimurium*/TA98, 100, 1535, 1537, and *E.coli* WP2 uvrA
Metabolic activation:	Yes; Rat liver, induced with phenobarbital and 5,6-benzoflavone
Concentration tested:	-S9 mix; 0, 0.195, 0.391, 0.781, 1.56, 3.13, 6.25 µg/plate (TA1537); 0, 0.781 - 50.0 µg/plate (TA100, TA1535, TA98 (Test 1)); 0, 0.391 - 12.5 µg/plate (TA1535 (Test 2)); 0, 0.781 - 25.0 µg/plate(TA100, TA98(Test 2)); 0, 156 - 5000 µg/plate(WP2 uvrA) +S9 mix; 0, 6.25 - 200 µg/plate (TA100, TA1535, TA98, TA1537); 0, 19.5 - 625 µg/plate (WP2 uvrA)
Remarks:	DMSO was used as a vehicle; Positive controls consisted of S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2 uvrA), Sodium azide (TA1535) and 9-Aminoacridine (TA1537); +S9 mix; 2-Aminoanthracene (all five strains). The test article was plated in triplicate with two replicates.
Results	
Result:	No positive responses were induced by Diisopropylbenzene in any of the tester strains
Cytotoxic conc.:	Toxicity was observed at 6.25 µg/plate (TA1535, TA1537) and 12.5 µg/plate (TA100, TA98), 5000 µg/plate (WP2 uvrA) without an S9 mix and at 100 µg/plate (TA100, TA1535, TA98, TA1537) and 500 µg/plate (WP2 uvrA) with an S9 mix.
Precipitation conc.:	No precipitate was noted in the report.
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Statistical methods:	Unknown
Remarks:	
Conclusions	Material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	This summary was obtained from the below referenced website. While the summary for this study was relatively scant and it is unknown what year it was conducted, it was still noted to have followed established OECD guidelines and GLP assurances.
References	Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-0025, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627 (http://wwwdb.mhw.go.jp/ginc/)
Other	

Test Substance	
Test substance:	DIPB Feedstock / Cumene Tower Bottoms
Remarks:	The purity of the material utilized in this study is unknown, as it is a complex mixture. However, historically this mixture has contained 25-40% mixed DIPB isomers.
Method	
Method:	Other
Test type:	CHO/HGPRT mutagenicity test
GLP:	Yes
Year:	1985
Species and Cell Type:	Chinese Hamster Ovary cells, cell line CHO-K1
Metabolic activation:	Yes; Aroclor 1254-induced rat liver S9 fraction
Concentration tested:	8-50 ug/ml (-S9) and 32-150 ug/ml (+S9)
Remarks:	The methods followed in this study are essentially identical to those used in OECD TG-476 and outlined by O'Neill and Hsie (1979). Negative controls were medium and vehicle each was +/- S9. Pluronic F127 was mixed in a 1:1 ratio (w/w) with absolute ethanol. The final concentration of F127 in the dosing preparation was 6% and 0.04% in the culture medium. Positive control chemicals were benzo(a)pyrene with S9 and ethyl methanesulfonate (each in test substance vehicle). An initial toxicity assay was performed +/-S9 activation at concentrations ranging from 8 to 5,000 ug/ml. The dosing regimen for the mutagenesis assay was designed to produce >10% survival. Sufficient cells were seeded to treatment flasks (3 per group) on Day 1 to give approximately 1 million cells on Day 2. On Day 3, all cultures were checked for evidence of cytotoxicity, and those which showed either excessive to no toxicity terminated. Cultures from 4 test substance dose groups were subcultured. Two hundred cells were added to each of four 60 mm cytotoxicity plates. These were incubated, fixed, and stained. Routinely, 10^5 - 10^6 cells were also seeded to a 100 mm dish on Day 3. These expression cultures were subcultured 3 times, the last on Day 10. At that time, 200 cells were seeded to each of 4 viability plates as above, and 2 x 10^5 cells seeded to each of 5 mutagenicity plates in selective medium. The cultures were reincubated undisturbed until Day 17 when they were fixed and stained.
Results	
Result:	Cloning efficiency at the time of mutant selection was not significantly decreased for test substance groups with activated treatment. After non-activated treatment, cloning efficiency at the time of mutant selection was slightly decreased for all test substance dose groups. However, after statistical analysis using the MUTANT program, there was neither a dose-related response nor a significant increase in the number of mutants after S9 activated or non-activated treatment with DIPB feedstock.
Cytotoxic conc.:	128 ug/ml (and higher) with S9 and 32 ug/ml (and higher) minus S9.
Genotoxic effects:	
With activation	Negative
Without activation	Negative

Statistical methods:	Statistical analysis was conducted using the MUTANT program. A test is considered positive if there is a significant ($p < 0.05$) increase in mutant colonies at any dose level and a dose-related response. It is considered negative if neither criterion is met. If only one criterion is met, the test results are considered equivocal.
Remarks:	
Conclusions	Material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	Actual percentage of DIPB in test article is unknown.
References	Gulf Oil Products Co.; GLSC 84-2120 (Document date: 4/18/85) O'Neill, J.P. and A.W. Hsie. 1979. The CHO/HGPRT Mutation Assay: Experimental Procedures. Banbury Report 2:55-63.
Other	

D. Genetic Toxicity – Chromosomal Aberrations

Test Substance	
Test substance:	Diisopropylbenzene
Remarks:	Purity was 99.8%. Material is a variable composition of ortho, meta, and para isomers.
Method	
Method:	OECD: TG-473
Test type:	*In vitro* chromosomal aberration
GLP:	Yes
Year:	Unknown
Species/strain:	Chinese hamster lung cells
Route of exposure:	*In vitro*
Concentration tested:	-S9 mix (continuous treatment): 0, 0.0038, 0.0075, 0.015 mg/mL -S9 mix (short-term treatment): 0, 0.0019, 0.0038, 0.0075 mg/mL +S9 mix (short-term treatment): 0, 0.030, 0.060, 0.12 mg/mL
Metabolic activation:	Yes; Rat liver, induced with phenobarbital and 5,6-benzoflavone
Remarks:	Acetone was used as a vehicle; positive controls consisted of Mitomycin C (-S9 mix) and Cyclophosphamide (+S9 mix). Study was conducted in replicate.
Results	
Result:	No evidence of clastogenicity or polyploidy was seen under the conditions of this experiment.
Cytotoxic conc.:	None indicated
Precipitation conc.:	None indicated
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Statistical methods:	Unknown
Remarks:	
Conclusions	Test material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	This summary was obtained from the below referenced website. While the summary for this study was relatively scant and it is unknown what year it was conducted, it was still noted to have followed established OECD guidelines and GLP assurances.
References	Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano-shi, Kanagawa, 257-0025, Japan. Tel +81-463-82-4751 Fax +81-463-82-9627 (http://wwwdb.mhw.go.jp/ginc/)
Other	

Test Substance	
Test substance:	DIPB Feedstock / Cumene Tower Bottoms
Remarks:	The purity of the material utilized in this study is unknown, as it is a complex mixture. However, historically this mixture has contained 25-40% mixed DIPB isomers.
Method	
Method:	Other
Test type:	Mouse Micronucleus Assay
GLP:	Yes
Year:	1985
Species/strain:	Swiss Mice/Crl:CD-1 (ICR) BR
Sex:	Both
Route of exposure:	Oral
Doses:	1.25, 2.5 and 5 g/kg
Statistical methods:	Student's t-test for differences between treated groups and vehicle control.
Remarks:	The methods followed in this study are essentially identical to those prescribed in OECD TG-474. Ten mice of each sex were administered doses of 0, 1.25 and 5.0 g/kg of DIPB and paraffin oil (negative control) for 2 consecutive days. Ten mice of each sex were also administered 5.0 g/kg of DIPB and paraffin oil for 1 day. Four mice of each sex were administered a single dose by ip injection of the positive control chemical, cyclophosphamide. Animals receiving a single test material and negative control dose were sacrificed on Days 2, 3 and 4; animals receiving 2 doses were sacrificed on Days 3 and 4, and animals given the positive control were sacrificed on Day 3 only. Smears of blood and bone marrow were prepared and stained for observation.
Results	
Effect on PCE/NCE ratio:	No significant effects were noted in this ratio.
Genotoxic effects:	No statistically significant changes in the incidence of micronuclei in polychromatic erythrocytes were seen.
Remarks:	No mortalities were observed in the range finding study. However, 1/10 males and 2/10 females receiving 2 doses of 5 g/kg died between Day 0 and 4.
Conclusions	Material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	Actual percentage of DIPB in test article is unknown.
References	Gulf Oil Products Co.; GLSC 84-2121 (Document date: 4/24/85)
Other	

E. Genetic Toxicity – Primary DNA Damage

Test Substance	
Test substance:	DIPB Feedstock / Cumene Tower Bottoms
Remarks:	The purity of the material utilized in this study is unknown, as it is a complex mixture. However, historically this mixture has contained 25-40% mixed DIPB isomers.
Method	
Method:	Other
Test type:	In Vitro Unscheduled DNA synthesis (UDS) in primary rat hepatocyte cultures
GLP:	Yes
Year:	1985
Species/strain:	Rat/Fischer 344
Concentration tested:	4, 8, 16, 32, 64, 128, 256 and 512 ug/ml
Control groups:	Vehicle control (Pluronic F127 Polyol); Positive control (2-Acetoaminofluorene) and negative control.
Statistical methods:	The test substance was considered positive for unscheduled DNA synthesis when the mean net nuclear grain count exceeded that of the concurrent negative control by at least 6 grains per nucleus. Fisher Exact Test and chi Square Analysis were also used to compare percentage of cells in repair between the test substance and the negative control.
Remarks:	The methods followed in this study are essentially identical to those prescribed in OECD TG-482. The F127 was diluted 1:1 by weight with absolute ethanol. This 50% solution was used to emulsify the test substance at a concentration of 22% F127 in the dosing preparation. The dosing preparation was added to 5 ml cultures in 50 ul aliquots producing a culture concentration of 0.22% F127. Primary rat hepatocytes were derived from freshly perfused rat liver (1 male, 10 weeks of age, 206 g BW). Cultures were seeded with 2 x 10^5 cells/ml on Day 1. Three cultures per group were exposed to ^{3}H-thymidine and test substance for 19 hours. Cells growing on cover slips were rinsed, fixed, air-dried, and glued to microscope slides on Day 2. On Day 3, the slides were dipped in autoradiographic emulsion and stored in the dark at 2-8^{o}C. Autoradiographs were developed and stained on Day 10.
Results	
Result:	No increases in DNA synthesis above the negative control were noted.
Cytotoxic conc.:	DIPB Feedstock was toxic to primary hepatocytes at 64 ug/ml and higher. The positive, vehicle, and negative controls gave expected responses for unscheduled DNA synthesis.
Genotoxic effects:	Negative
Remarks:	
Conclusions	Material did not induce DNA synthesis under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	Actual percentage of DIPB in test article is unknown.
References	Gulf Oil Products Co.; GLSC 84-2122 (Document date: 2/25/85)
Other	

F. Genetic Toxicity – Other (Cell Transformation)

Test Substance	
Test substance:	DIPB Feedstock / Cumene Tower Bottoms
Remarks:	The purity of the material utilized in this study is unknown, as it is a complex mixture. However, historically this mixture has contained 25-40% mixed DIPB isomers.
Method	
Method:	Other
Test type:	Cell Transformation Assay in BALB-3T3 Mouse Embryo Cells
GLP:	Yes
Year:	1984
Cell type:	Mouse embryo cells/BALB-3T3-A31-1-1
Concentration tested:	5, 25, 60, and 90 ug/ml.
Remarks:	Each treatment group consisted of 15 cultures for cell transformation and 2 cultures for colony formation. Controls cultures received 0.22% Pluronic F127 Polyol medium or 3-methylcholanthrene (1 ug/ml with 0.22% Pluronic F127 Polyol). The F127 was diluted 1:1 by weight with absolute ethanol. This 50% solution was used to emulsify the test substance at a concentration of 22% F127 in the dosing preparation. The dosing preparation was added to 5 ml cultures in 50 ul aliquots producing a culture concentration of 0.22% F127. Transformation cultures were seeded with approximately 1 x 10^4 cells and colony formation cultures with approximately 100 cells on Day 1. The cultures were exposed to the test substance for 2 days, beginning on Day 2. The medium was changed on all cultures on Day 4. Colony formation cultures were fixed and stained for colony counting on Day 10. The medium was changed weekly on all transformation cultures. Fixation and staining of transformation cultures for focus counting and evaluation were on Day 29. Colonies (at least 50 cells) in culture vessels were counted visually and, where required, examined microscopically. Foci in transformation cultures were counted visually and examined microscopically. The colony forming efficiency for each group and the relative colony forming efficiency were calculated.
Results	
Result:	No increases in cell transformations were noted in DIPB feedstock exposed cells. Expected responses were seen in all control groups.
Cytotoxic conc.:	Viability was 72% at 8 ug/ml, 35% at 64 ug/ml, 6% at 128 ug/ml, and 0% at higher concentrations.
Criteria for a Positive/Negative Test:	A test is considered positive if there were 1.) A two-fold increase in Type-III foci at the highest dose above that seen in negative control cultures, with or without a dose-related response, or 2.) A two-fold increase at two or more consecutive dose levels. Where negative control cultures have no Type-III foci, at least 2 foci would be needed for a dose level to be considered positive. A test is considered equivocal if a two-fold increase occurred at any one level other than the highest acceptable dose. A test is negative if none of the above applies.
Remarks:	While included under genetic toxicity this assay does not technically assess the affect of chemicals to damage chromosomes.

Conclusions	Test material did not induce increases in the number of transformed cells under conditions of this assay.
Data Quality	
Reliability:	2; Reliable with restrictions
Remarks:	Actual percentage of DIPB in test article is unknown.
References	Gulf Oil Products Co.; GLSC 84-2123 (Document date: 1/23/85)
Other	

G. Developmental Toxicity and Reproductive Toxicity

Narrative summaries of the several studies used to satisfy this endpoint can be found in Attachment I, entitled "The Use of Various Mono- and Di-Alkylbenzene Surrogates for the HPV Candidate Diisopropylbenzene Chemicals in SIDS Reproductive/Developmental Toxicity Testing" by Mr. James Schardein. In addition, summaries will be available in various other public documents that are in various stages of completion. These include the OECD SIDS dossiers for Cumene (isospropylbenze), ethylbenzene, and 1,4-diethylbenzene, as well as the summaries being prepared for the mixed isomers of diethylbenzene (CAS# 25340-17-4) through the ICCA HPV program.